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1995



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41025-24 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of the Cell Surface Receptor for IgE

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: Henry Metzger, MD Chief, Chemical Immunology Section ARB/NIAMS
Su-Yau Mao, PhD Visiting Scientist
Ute Kent, PhD IRTA Fellow
Victor Pribluda, PhD Visiting Scientist
Clara Pribluda, MD Visiting Fellow
Becky Vonakis, PhD IRTA
T Yamashita, PhD Visiting Fellow

COOPERATING UNITS (if any)

David Holowka, Barbara Baird, E.-Y. Chang (Cornell University)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Chemical Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N258, Bethesda, MD 20892

TOTAL STAFF YEARS:

6 2/3

PROFESSIONAL:

6 2/3

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The high affinity receptor for IgE on mast cells & basophils (Fc(epsilon)RI) plays a central role in immediate hypersensitivity reactions. Reaction of receptor-bound IgE with polyvalent antigen clusters the receptors & this stimulates cellular secretion of both preformed & newly synthesized mediators of inflammation. Our studies focus on the molecular mechanisms by which aggregation of the receptors generate these cellular responses. The similarity of Fc(epsilon)RI to other receptors of the immune system (e.g. the clonotypic receptors on T & B lymphocytes), make it likely that the significance of such studies extends beyond the IgE/mast cell system. During the past year, our principal progress was in two areas: 1) Completing the first phase of our studies on the association between a tyrosine kinase and the receptor. Using either gentle isolation procedures or chemical crosslinking, we found convincing evidence for the following mechanism by which the topological change induced by aggregation of the receptors is converted into a biochemical signal: a) Some unaggregated receptors are constitutively associated with the src-like tyrosine kinase--p55/56lyn in the case of receptors in the rat "RBL" cell-line with which we work. b) Aggregation of receptors in effect induces the formation of enzyme-substrate complexes so that the kinase on one receptor can (trans)phosphorylate other receptors newly approximated to it. c) Such phosphorylated receptors "recruit" additional kinase thereby initiating the biochemical cascade. 2) Further analysis on the dynamics of signal transduction in relation to aggregation of the receptors in vivo. Quantitative analysis of the relationship of aggregation to phosphorylation of receptor tyrosines revealed a discrepancy that cannot be accommodated by any simple model. The data suggest that not only the amount but also the rate of aggregation may influence the cellular response. These results have general implications on the role of ligand-receptor interactions in initiating alternative cellular responses.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 AR 41048-16 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Bacterial Cell Wall-Induced Arthritis in the Rat

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

R.L. Wilder Chief

Y. Du Visiting Fellow

E. Remmers Staff Scientist All IJDS, ARB, NIAMS

Lyn Ge IRTA Fellow

S. Kotake Visiting Fellow

COOPERATING UNITS (if any)

Developmental Endocrinology Branch, NICHD

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

We and others have shown that Lewis (Lew) rats are highly susceptible to a wide spectrum of autoimmune diseases such as bacterial cell wall arthritis. We have also demonstrated that they develop severe inflammation in response to nonspecific proinflammatory agents such as carrageenin. In contrast, Fischer (F344) rats develop minimal or no inflammation in response to the same stimuli. We have previously provided data suggesting that these differences are related to a global deficiency in hypothalamic-pituitary-adrenal axis (HPA) responsiveness in Lewis rats. We have continued to characterize the functional basis of the HPA axis defect and have now shown that Lewis and Fischer rats respond similarly to corticosteroids. These data render unlikely the hypothesis that the HPA axis defect in Lewis rats is a consequence of primary abnormalities in type 1 corticosteroid receptors or tissue sensitivity to corticosteroids.

We have also continued to characterize cytokine responses of Lewis and Fischer rats following LPS injection and have noted several prominent differences in these strains. These responses parallel the HPA differences. As an extension of these studies, we have also begun to clone genes that are differentially expressed in these strains in response to LPS. To date, we have cloned 2 novel transcripts (LF-1 and LF-2) and are sequencing these mRNAs. These data may provide important insights into the mechanisms regulating susceptibility and resistance to autoimmune inflammatory diseases.

DEPARTMENT OF STATE AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41066-13 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Characterization of synovial tissues from patients with RA and related conditions

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

R.L. Wilder Chief

E. Remmers Staff Scientist All IJDS, ARB, NIAMS

K. Kanik Med. Staff Fellow

S. Kotake Visiting Fellow

COOPERATING UNITS (if any)

Holland Labs, American Red Cross

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

1.5

CHECK APPROPRIATE BOXES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Previous studies from our laboratory have suggested that angiogenesis plays a major role in rheumatoid arthritis (RA). Related studies from our laboratory have indicated that enhanced expression of cyclooxygenase-2 (Cox-2) is also important and parallels the intensity of the inflammatory process and angiogenesis. We have now defined a potential biochemical mechanism that may link these observations. Interleukin-1 (IL-1), which is a powerful stimulator of Cox-2, and prostaglandin E2, which is product of Cox-2, induce the expression of vascular endothelial growth factor (VEGF) in rheumatoid synovial fibroblasts. VEGF expression is upregulated in rheumatoid synovium and probably plays a role in driving angiogenesis. These data may also provide insights into mechanisms by which corticosteroids or nonsteroidal inflammatory drugs regulate inflammatory angiogenesis. These agents have powerful suppressive effects on Cox-2 and prostaglandin E2 production. In addition, corticosteroids suppress interleukin-1 production.

Our laboratory is also characterizing cytokine transcription in synovial tissues from patients with early synovitis. Subjects include those with rheumatoid arthritis, reactive arthritis, a various other forms of arthritis. We have noted that the expression of tumor necrosis factor-alpha (TNF-alpha) and IL-1 in virtually all forms of early synovitis. Similarly, gamma interferon expression is common, whereas IL-4 and IL-13 expression is rare. These data suggest that macrophage-derived and type 1 lymphocyte-derived cytokines, in contrast to type 2 cytokines, predominate in early synovitis, regardless of the type of disease. The data suggest the cell-mediated, rather than humoral mechanisms play essential roles in the early stages of the inflammatory process in the joints.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41074-08 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies on etiology and pathogenesis of myositis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB,NIAMS

Nina Raben, M.D., Ph.D., Research Chemist

Ralph Nichols, Ph.D., Senior Staff Fellow

Elizabeth Adams, M.D., Clinical Associate

Jeffrey Sherman, M.D., Senior Staff Fellow

Laurie Beitz, M.D. IRTA Fellow

COOPERATING UNITS (if any)

Terry O'Hanlon, Ph.D., CBER/FDA

Dennis Klinman, M.D., Ph.D., CBER/FDA

Lisa Rider, M.D., CBER/FDA

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Building 10 Room 9N244, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.55

PROFESSIONAL:

2.55

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Idiopathic inflammatory myopathy (polymyositis, dermatomyositis, and related disorders) is a family of inflammatory diseases in which disease-specific autoantibodies occur and for which there is considerable indirect evidence pointing to a viral etiology. We have over the past several years, seen and studied and collected serum, blood, and muscle specimens from well over 500 patients suspected of having myositis and we have collected epidemiologic information on many patients. Our recent attention has been focussed on the mechanism of autoimmunity and on the details of tissue within the affected muscles.

We have completed studies on the primary sequence of two more of the target autoantigens, isoleucyl- and (in collaboration with Paul Schimmel at MIT) alanyl-tRNA synthetase and have identified the chromosomal location of four of the target aminoacyl-tRNA synthetases. We have produced pure recombinant histidyl-tRNA synthetase (HRS) and, with Craig Hyde, made large crystals, but the x-ray diffraction studies have so far been of only low resolution. Dr. Terry O'Hanlon and Dr. Fred Miller of CBER/FDA with Dr. Raben, have identified a transcript of what appears to be the mitochondrial HRS.

We have studied the cytokines and chemokines produced by circulating leukocytes (with Dr. Dennis Klinman of CBER/FDA) and by cells within affected muscle biopsies, and we have continued preparations to study the peptides found in Class I HLA antigens on the surface of myoblasts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41076-08 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Therapeutic trials in idiopathic inflammatory myopathies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

PI: Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section, ARB,NIAMS

Jeffrey Sherman, M.D., Senior Staff Fellow

Frederick Miller, M.D., Ph.D., Special Volunteer

Elizabeth Adams, M.D., Clinical Associate

Lourdes Villalba, M.D., Clinical Associate

COOPERATING UNITS (if any)

Jeanne Hicks, M.D., CC Rehabilitation

Melissa Bartlett, M.S., CC Radiology

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

1.55

PROFESSIONAL:

1.55

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In an attempt to find better ways to treat myositis, the group has explored the use of both established medications used in new ways or combinations or new therapies. These trials are virtually the only such trials of the treatment of this very uncommon condition and thus also serve to draw patients to the Clinical Center for the other research projects carried out by the group on the basic mechanisms of myositis and related or mimicking diseases (outlined in ZO1 AR 41074-08 and ZO1 AR 41099-03).

A randomized, crossover trial of high dose intravenous methotrexate with leucovorin rescue and of a combination of oral methotrexate and azathioprine has been completed in the past year and an analysis of the results is being prepared for publication.

We have begun a trial of the purine analogue, fludarabine, in the therapy of myositis. A quantitative method of assessing muscle inflammation in myositis has been developed and will be used in current and future therapeutic trials. Thirteen of the planned sixteen patients have entered the trial so far, of whom almost half have completed the six-month treatment phase.

A trial of the anti-thyroid drug, methimazole, will be begun in the next year. This agent was chosen because it may alter the ability of muscle cells to serve as a target for immunologic attack. In this past year, the methods for assessing the effect of the drug on muscle and blood cells have been developed in collaboration with NIDDK & NCI.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41083-06 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Familial Mediterranean Fever

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
Daniel L Kastner, MD, PhD, Chief, Genetics Section, ARB/NIAMS

Ivona Aksentijevich, MD, IRTA Fellow

Christopher Mojck, MD, PhD, Staff Fellow, LI/NIAD

Xiang Chen, MD, IRTA Fellow

Pu Liu, MD, PhD, Senior Staff Fellow, LGT/NCHGR

Michael Centola, PhD, IRTA Fellow

Trevor Blake, Biologist, LGT/NCHGR

Ernesto Levy, MD, Visiting Associate

Raman Sood, PhD, IRTA Fellow

Geryl Wood, Biologist

COOPERATING UNITS (if any)

LGT/NCHGR; LI/NIAD; Heller Institute, Israel; Cedars-Sinai Hosp, Los Angeles; Los Alamos Natl Lab; Women's and Children's Hosp, Adelaide; Dept Anatomy, U Michigan; Whitehead Institute, MIT

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1820

TOTAL STAFF YEARS:

7.0

PROFESSIONAL:

4.0

OTHER:

3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.)

Familial Mediterranean fever (FMF) is characterized by episodes of fever with abdominal pain, pleurisy, and/or arthritis; some patients also develop systemic amyloidosis, leading eventually to renal failure. FMF is inherited as a single autosomal recessive gene (designated MEF), which is very common among Jewish, Armenian, Turkish, and Arab populations. Since the biochemical lesion in FMF is unknown, our goal is to identify the FMF gene by positional cloning. By the beginning of this year, we had narrowed the candidate interval to a region of approximately 1 Mb on the short arm of chromosome 16. In 1994-95 our efforts were divided among a) genomic cloning of the candidate interval; a) further genetic refinement of the interval; c) transcript identification; and d) functional studies of FMF leukocytes.

Genomic cloning began with the identification of CEPH megaYACs. However, due to the instability of megaYACs from this part of the genome, a major effort in the last year has been directed towards developing a cosmid contig. Using the Los Alamos 10X chromosome 16-specific cosmid library, we have constructed a 525 kb contig on the telomeric end of the 1 Mb candidate interval, and a 350 kb contig on the centromeric end. We have made NotI and EcoRI restriction maps of these contigs. We are attempting to close the gap between the two contigs with a combination of cosmids, YACs, PACs, BACs, and P1s.

Refined genetic mapping was facilitated by our identification of 4 new highly polymorphic microsatellites from the cloned genomic DNA. These new markers exclude an additional 250 kb on the telomeric end of the interval, and 300 kb on the centromeric end of the interval. Moreover, at one of the 4 marker loci there are no recombinants among a panel of 65 Jewish, Armenian, and Arab families. A specific allele of this latter locus is in strong linkage disequilibrium with FMF among North African Jewish FMF patients; Luria-Delbruck analysis of linkage disequilibrium data in the Moroccan Jewish founder population places MEF within about 80 kb of this marker.

We are identifying transcripts from the FMF candidate interval by exon amplification from cosmids. To date, we have isolated a total of 28 putative exons from the region, and we are screening cDNA libraries for the corresponding full-length transcripts. In parallel, collaborators in Australia are using direct cDNA selection on cosmid pools to identify additional transcribed sequences.

Phenotypic analysis of FMF leukocytes may facilitate analysis of positionally-defined candidate genes; over the last year we have confirmed that leukocytes from FMF patients show increased adhesion to fibronectin and type I collagen, but express normal levels of known cell-surface adhesion molecules.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41088-05 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic and Neuroendocrine Factors in the Autoimmune Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

R.L. Wilder Chief, IJDS

K. Kanik Medical Staff Fellow, IJDS

K. Kalogeras Special Volunteer

COOPERATING UNITS (If any)

Developmental Endocrinology Branch, NICHD
Leslie Crofford, University of Michigan

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1

PROFESSIONAL:

1

OTHER:

1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

Studies from animal models have suggested the possibility that hypothalamic-pituitary-adrenal axis (HPA) and other neuroendocrine dysfunctions may play a role in rheumatoid arthritis (RA) and other autoimmune conditions. For these and other reasons, we are conducting studies investigating neuroendocrine function in patients with RA and related arthropathies. We have completed one study that supports our view that adrenal hormone production is blunted in patients with RA. We investigated circadian secretion of ACTH, cortisol, interleukin-1 (IL-1) and IL-6 and demonstrated that RA patients show "normal" or occasionally low 24-hour cortisol production, while plasma IL-6 levels are markedly elevated. Of interest, we noted that IL-6, like ACTH and cortisol, is secreted with a circadian pattern, and the pulses of cortisol are linked to pulses of IL-6, with IL-6 leading cortisol by about 1 hour. Linkage of IL-6 secretion to ACTH secretion was much weaker. These data suggest that IL-6 may directly stimulate adrenal secretion of cortisol, but, more importantly, the data support our hypothesis that adrenal cortisol production in RA patients is "inappropriately normal" or blunted. We would expect markedly increased cortisol production the setting of high IL-6 levels. Additional data have been collected on adrenal androgen levels in new-onset RA patients. Interestingly, the production of adrenal androgens, such as dehydroepiandrosterone (DHEA) and (DHEA-S), is even more abnormal than cortisol. These data indicate that adrenal hypofunction in RA patients is not restricted to cortisol but suggest more global neuroendocrine dysfunction.

Initial studies of HPA axis and sympatho-adrenal function in patients with fibromyalgia were completed. These patients exhibit blunted HPA responses to corticotropin releasing hormone (CRH) and strikingly low levels of neuropeptide Y. These data suggest abnormalities in the two major components of the stress response system may play a role in this perplexing condition.

We also completed a study testing the hypothesis that corticosteroids mediate some of their peripheral antiinflammatory effects by stimulating somatostatin, a well-known antiinflammatory peptide. In an animal model of aseptic inflammation, we noted increased local expression of somatostatin following corticosteroid treatment in concert with suppression of local proinflammatory substances such as substance P, CRH and tumor necrosis factor-alpha.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41092-04 ARB

PERIOD COVERED

October 1, 1994 to September, 1994

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mapping of Genes and Genetic Polymorphisms in Rats

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

R.L. Wilder Chief
E. Remmers Staff Scientist
Y. Du Visiting Fellow All IJDS, ARB, NIAMS
L. Ge IRTA Fellow
S. Kotake Visiting Fellow
R. Longman PreIRTA Fellow
A. O'Hare Howard Hughes Medical Scholar

COOPERATING UNITS (if any)

M. Griffiths, Univ. of Utah

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Inflammatory Joint Diseases Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5

PROFESSIONAL:

5

OTHER:

5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Rats are an important experimental model for many human diseases, many of which have a genetic component. As another approach to investigating fundamental origins of autoimmune diseases, we have initiated studies with the ultimate goal of mapping genes regulating autoimmune disease in rats. A initial and critical step to meet this objective is the development of a genetic linkage map applicable to autoimmune-disease prone and -resistant rat strains. Over the past year, we have identified more than 300 new polymorphic DNA markers that distinguish various inbred rat strains (total of about 900 since the initiation of the project). We have now mapped a large fraction of these markers in F2 progeny of LEW x F344 (more than 400 markers total), LEW x BN (more than 400 markers), or DA x F344 (about 200 markers).

The genetic map that we have developed has been used the refine our previous localization of the rat athymic nude trait on rat chromosome 10. We have also now refined our localization of the rat osteopetrosis gene (op) to the distal end of rat chromosome 10.

Several efforts are in progress to map phenotypes related to autoimmune disease in F2 progeny of LEW X F344 rats. In addition, we are mapping genes that regulate collagen-induced arthritis (CIA), an autoimmune-disease model that resembles rheumatoid arthritis, in F2 progeny of DA x F344 rats. Over the past year, we have generated data in the LEW X F344 progeny indicating that virtually all of the phenotypes under investigation are subject to complex regulation involving 2-4 genes in distinct chromosomal locations. In the CIA model, we have identified a major regulatory locus (LOD approximately 63), linked to the major histocompatibility complex (MHC) on chromosome 20. This locus acts dominantly and contributes approximately 50% to the phenotypic variability. In addition, at least 4, and possibly 5, additional chromosomal regions (chromosomes 1,4,7 and 14) contribute to phenotypic variability. The mode of action of the non-MHC genes is still being analyzed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41095-04 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Translational and rotational power terms in 6 degree-of-freedom joint modeling

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
J.P. Holden, IRTA Fellow, Biomechanis Laboratory, ARB/NIAMS
S.J. Stanhope, Director, Biomechanics Laboratory (BML), RMD/CC

COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)
T.M. Kepple, Programmer Specialist, BML, RMD/CC K.L. Siegel, Senior Staff Therapist, BML, RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was inactive during the period covered. Separate but related projects were undertaken to better understand the source(s) of the joint translations previously measured at the ankle and knee.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41096-04 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Nonequilibrium in the translation equations of motion

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
J.P. Holden, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS
S.J. Stanhope, Director, Biomechanics Laboratory, RMD/CC

COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This project was inactive during the period covered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41099-04 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The genetic basis for metabolic myopathies

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**

PI: Nina Raben, M.D., Ph.D., Research Chemist, ARB,NIAMS

Paul H. Plotz, M.D., Chief, Connective Tissue Diseases Section

Ralph Nichols, Ph.D., Senior Staff Fellow

Elizabeth Adams, M.D., Clinical Associate

Joseph Zaretsky, M.D., NRC Fellow

Jeffrey Sherman, M.D., Senior Staff Fellow

Rachel Exelbert, Biologist

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Connective Tissue Diseases Section

INSTITUTE AND LOCATION

NIAMS, Building 10 Room 9N244, Bethesda, MD 20892

TOTAL STAFF YEARS:

3.1

PROFESSIONAL:

3.0

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☐ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

In the course of studying autoimmune mechanisms in myositis, an inflammatory muscle disease, a number of patients with other muscle disease have been evaluated. Two genetic metabolic myopathies, phosphofructokinase (PFK) deficiency and acid maltase (GAA) deficiencies, have been selected for studies at the molecular level.

The studies of PFK deficiency, now drawing to a close, have focussed on defining the genetic abnormalities in different patient populations, and a number of mutations have been found. Experiments in collaboration with a laboratory in Germany have used a yeast expression system to study the biochemical properties of the abnormal enzymes.

Studies of GAA deficiency have centered on the adult form of the disease. An infantile form is fatal in the first year of life. Studies on the mechanism of a common GAA mutant genotype, shared by 70% of adult-onset patients, have been expanded in a model system. This system enabled us to identify a transcriptional silencer in the GAA gene, which has implications for gene therapy. Experiments to lay the ground work for therapeutic gene transfer with a recombinant retrovirus bearing the normal cDNA resulted in the restoration of GAA activity in affected fibroblasts. Similar experiments with affected myoblasts are ongoing. Plans are underway for developing a GAA deficient mouse model for in vivo gene therapy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41100-03 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Skeletal tracking with skeletal fixation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
J.P. Holden, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS
M.C. Brown, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS

COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH (L.H. Gerber)
S.J. Stanhope, Director, Biomechanics Laboratory, RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.7

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Quantitative gait analysis requires attachment of optical targets to the skin's surface. Data derived from monitoring the locations of these targets while a patient walks provide the basis for an objective study of the movement. The movement of the skin and underlying soft tissues relative to the bone is known to be a source of error in the measurements, but the magnitude of the errors is unknown and goes uncorrected in standard analyses. The purpose of this study is to evaluate the magnitude and pattern of error produced by tracking the skeletal system using surface mounted targets.

As a subject walks in the laboratory, a video-based target tracking system is used to measure simultaneously the movement of two sets of reflective targets attached to each body segment being studied -- one set mounted to the surface (standard technique) and one mounted to the bone. Skeletal fixation of the bone-mounted targets is accomplished using a percutaneous skeletal tracker (PST). Each target set is used separately in calculations of segment motions (kinematics) and the net joint forces and moments (inverse dynamics analysis) that cause the motions. The differences in results represent the errors introduced by the surface target attachment technique. Inverse dynamics analyses were performed on data from three subjects who walked with the PST attached to the shank (lower leg). The magnitudes of the differences in forces and moments would not likely affect the clinical interpretation of the data, but may be important for other applications such as musculoskeletal modeling. Additional data were also collected to measure the motion with respect to bone of a single surface mounted target on the lateral aspect of the knee. The motion of this target (used by some laboratories to estimate the location of the knee joint center) was compared with the joint center estimate derived from the set of surface mounted targets at the mid-shank. The movement of the single lateral knee target with respect to bone during gait was more than twice that of the knee joint center estimated from the surface mounted targets.

The PST is currently undergoing modification and cadaveric testing to establish a design and attachment technique for use on the femur.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41101-02 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Intracellular Signaling by the Cell Surface Receptor for IgE

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: Juan Rivera, Ph.D. Senior Staff Fellow, Signal Transduction Group,
Section on Chemical Immunology, ARB, NIAMS
En-Yuh Chang, Ph.D. IRTA Fellow
Hana Haleen-Smith Chemist
James S. Song, Ph.D. IRTA Fellow

COOPERATING UNITS (if any)

LCCTP, NCI (Z. Szallasi and P.M. Blumberg)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Chemical Immunology Section

INSTITUTE AND LOCATION

NIAMS, Building 10, Room 9N252, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX (ES)

- ☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The molecular mechanisms that lead to the expression of newly synthesized mediators of inflammation are the central focus of our studies. We utilize a mast cell model, RBL-2H3 cells, due to the robust production of both inflammatory and immunoregulatory cytokines and the induction of their synthesis via the well characterized high affinity receptor for IgE (FcEpsilonRI). In particular the steps involved in the signaling pathway, beginning with IgE-antigen activation of the high affinity receptor for IgE and resulting in the induction of cytokine genes, are being explored. In the past year we have focused principally on the regulation of kinases, receptor adapter molecules and transcription factors known to be critical for the induction of gene expression. Three principal areas of investigation are underway: 1) Studies on the regulation of molecules purported to link cell surface activation of receptors with induction of gene expression. 2) Exploring the regulation of receptor-associated protein kinase C (PKC) delta in response to aggregation of the high affinity receptor for IgE. 3) Development of stably transfected mast cell lines over-expressing the individual isozymes of PKC to enable studies on cellular targets of enzyme activity.

Our new results show: 1) The calcium independent PKC-delta is tyrosine phosphorylated in response to receptor aggregation. This tyrosine phosphorylation of PKC delta inhibits its ability to recognize the receptor gamma chain as a substrate, but does not inactivate the enzyme since it readily phosphorylates other substrates. This result suggests a novel intracellular mechanism for regulation of substrate recognition. 2) In collaborative studies we have identified the site of phosphorylation as tyrosine 52 in the regulatory domain of PKC delta. 3) Studies on the regulation of Vav, a protein thought to play a role in MAP kinase activation and therefore gene transcription, show that this protein which we have found to associate with the FcEpsilonRI exists as a multicomponent complex in association with the adaptor molecule Grb2, the serine/threonine kinase Raf-1 and the p42 MAP



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41102-03 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Cystinuria

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
 Daniel L Kastner, MD, PhD, Chief, Genetics Section, ARB/NIAMS
 Elon Pras, MD, Visiting Fellow, Genetics Section, ARB/NIAMS
 Nina Raben, MD, PhD, Research Chemist, Connective Tissue Diseases Section, ARB/NIAMS

COOPERATING UNITS (if any)

Connective Tissue Diseases Section, ARB/NIAMS
 Heller Institute, Tel-Hashomer, Israel

Hadassah Medical Center, Jerusalem
 Beilinson Medical Center, Israel

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1820

TOTAL STAFF YEARS:

1.5

PROFESSIONAL:

1.5

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Cystinuria is an autosomal recessive disease in which there is excessive excretion of cystine in the urine, with precipitation of urinary cystine stones. Patients with this disorder may have renal colic, urinary tract obstruction, secondary urinary tract infection, or, if untreated, renal insufficiency. By the beginning of this reporting period we had mapped a cystinuria susceptibility gene to the short arm of chromosome 2, and had identified mutations in the SLC3A1 gene (which is located in the appropriate chromosomal region) in one American cystinuria family. Another group had independently identified 6 other SLC3A1 mutations in cystinuria. During the last year our goals have been to screen our panel of 17 cystinuria families for additional mutations, and to elucidate the genomic structure of SLC3A1.

Including the 2 mutations reported last year, we have now identified a total of 5 cystinuria-associated SLC3A1 mutations. This includes: a) a frameshift mutation and b) a large deletion in a non-Jewish American family; c) a C-to-T nonsense mutation at cDNA position 808 in 1 Israeli Druze and 4 Ashkenazi Jewish families (this mutation was found in 8 of 8 Ashkenazi carrier chromosomes examined); d) a C-to-A transversion at cDNA position 383 in a Persian and a Yemenite Jewish family; and e) an A-to-G transition in the splice donor site of exon 4, causing skipping of this exon, in a Moslem Arab family.

Studies of the genomic structure of SLC3A1 were based on our identification a P1/PAC contig from the appropriate chromosomal region. SLC3A1 contains 10 exons, and spans a total of approximately 45 kb of genomic DNA. We have constructed an EcoRI restriction map of the region, and have developed PCR primers from introns that allow exon amplification from genomic DNA, thus obviating the need for RT-PCR on illegitimate transcripts from lymphoid cells. We have also sequenced 650 bp upstream from the initiation codon, and have identified several regulatory elements in the promoter region.

Finally, we have obtained evidence that, in several Libyan Jewish families, the cystinuria susceptibility gene is not SLC3A1. This is based on the following observations on these families: a) for 5/5 individuals tested (3 of them from inbred families), we were unable to find mutations in the SLC3A1 cDNA; b) by urine amino acid analysis, we have confirmed the affection status of all individuals; c) using an intragenic microsatellite that we identified, we have found definite SLC3A1 recombinants; and d) hap-lotype analysis with other newly identified flanking genetic markers is also incompatible with SLC3A1 causing cystinuria in these families. Future studies will be directed toward the mapping and eventual cloning of a second cystinuria susceptibility gene.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41103-03 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetics of Rheumatoid Arthritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
Daniel L Kastner, MD, PhD, Chief, Genetics Section, ARB/NIAMS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Genetics Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892-1820

TOTAL STAFF YEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project was inactive during the reporting period.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41104-03 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies of the Rheumatic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

PI: J. H. Klippel Clinical Director, NIAMS
M.F. Gourley Senior Staff Fellow, ARB/NIAMS
D. Boumpas Visiting Scientist, ARB/NIAMS
D. Scott Special Volunteer, ARB/NIAMS
J. Pando Medical Staff Fellow, ARB/NIAMS
C. Yarboro Research Nurse, ARB
V. Gluck Pre-Doctoral IRTA

COOPERATING UNITS (if any)

NIDDK DD (R Sallie); Pharmacy, CC (F. Pucino); Washington University (J. Lefkowitz); Hospital for Special Surgery (JE Salmon); CDER/FDA (D. Klinman)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

4.0

PROFESSIONAL:

2.0

OTHER:

2.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are conducting a series of clinical investigations involving patients with systemic lupus erythematosus in which questions related to disease pathogenesis, epidemiology, therapy and co-morbidities are addressed. Therapeutic studies in progress include a long-term, randomized trial of corticosteroids and immunosuppressive drugs in patients with severe, proliferative lupus glomerulonephritis and a phase I/II of the nucleoside analog 2-chlorodeoxyadenosine similarly in patients with lupus nephritis. Studies are underway to assess risk factors associated with the development of osteoporosis and vascular thrombotic events. Collaborative studies with scientists interested in lupus pathogenesis during the past year have included the influences of cytokines in autoimmunity (D. Klinman), antibody binding to chromatin adherent to the collagen of the glomerular basement membrane (JB Lefkowitz), and FcγRIIIa alleles as risk factors for nephritis in African-Americans (JE Salmon).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41105-02 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Effect of Walking Speed on the Mechanics of Gait

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
J.P. Holden, IRTA Fellow, Biomechanics Laboratory, ARB/NIAMS

COOPERATING UNITS (if any)

Rehabilitation Medicine Department, Clinical Center, NIH
S.J. Stanhope, Director, Biomechanics Laboratory, RMD/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.7

PROFESSIONAL:

0.5

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Neuromuscular function and adaptation are often evaluated by comparing a patient's kinematic (movement) and kinetic (forces and moments) gait patterns with those obtained from healthy individuals. A feature of many patients' gait is a much slower than normal walking speed. It is important when interpreting a patient's data to distinguish which variations from normal are due solely to differences in walking speed and which are due to neuromuscular abnormalities or adaptations other than speed. The purpose of this project is to investigate the effect of dramatically different walking speeds on the mechanics of gait. One specific objective is to determine if a "quadriceps avoidance" pattern of net knee flexion-extension moments (reported in the literature for patients with anterior cruciate ligament deficiency) is demonstrated by healthy subjects at slower walking speeds.

Movement and force data have been collected from 18 healthy subjects (9 male, 9 female, ages 21-37 yrs) who walked at 25%, 50%, 75%, 100%, and 125% of a normalized speed of 0.785 statures/s ($\pm 2.5\%$). Speed was measured over a 2.28 m distance using two photocells and a gait speed indicator circuit. Inverse dynamics analysis was performed to calculate the net flexion-extension moments at the knee during the stance phase of gait.

Two-thirds of individual subjects had knee flexion-extension moment patterns that were similar across all five walking speeds. One-third of the subjects, however, demonstrated a quadriceps avoidance strategy (net flexor moment or zero moment) in at least one trial at the slowest speed. Decreased moment amplitude was related to decreased knee flexion during the "loading response" phase in early to mid stance. The subjects who produced the quadriceps avoidance pattern at the slowest speed did so by keeping the knee near full extension throughout the stance phase of gait.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
 ZO1 AR 41106-01 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemistry of Lymphocyte Activation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**

PI: **John J. O'Shea, M.D.**

Chief, Lymphocyte Cell Biology Section, ARB/NIAMS

James Johnston, Ph.D.

Senior Staff Fellow

Yi-Qing Chen, Ph.D.

Courtesy Fellow

Mary C. Riedy, Ph.D.

IRTA Fellow

Christopher Bacon

Courtesy Fellow

Masaru Kawamura, M.D.

Visiting Fellow

Aaron Davis

Laboratory

Technician

Eric Hanson

Pre-IRTA Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Lymphocyte Cell Biology Section

INSTITUTE AND LOCATION

NIAMS, Building 10 Room 9N262, Bethesda, MD 20892-1820

TOTAL STAFF YEARS:

7

PROFESSIONAL:

4.75

OTHER:

2.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Interleukin 2 and other cytokines dramatically influence the immune response and therefore understanding the molecular basis of cytokine signal transduction should provide important insights into lymphocyte activation. To this end, we cloned a new protein tyrosine kinase, termed JAK3 and showed that it is a critical element in signalling via the subfamily of cytokine (IL-2, IL-4, IL-7, IL-9, and IL-15) receptors that use the common γ chain (γc). We further showed that whereas JAK3 binds γc , JAK1 binds the IL-2R β . X-linked severe combined immunodeficiency (X-SCID) is due to mutations of γc . We demonstrated that several γc mutations result in the failure of this subunit to bind JAK3, whereas reconstitution of cell lines with γc mutations with the wild type allele restores the ability to activate JAK3 and JAK1. The disease X-linked combined immunodeficiency (X-CID) is also due to a mutation of γc . This allele binds JAK3 but does so at much reduced efficiency and this binding correlates with the reduced severity of this immunodeficiency. In addition, we have also now identified patients with SCID who lack JAK3 indicating that JAK3 and its function appear to be critical in the development of the immune system.

We have also investigated cytokine activated transcription factors, in particular STAT protein which are tyrosine phosphorylated transcription factors that contain SH2 domains. We have shown that IL-2 activates STAT3 and STAT5. IL-12 is a cytokine that has profound effects on T lymphocyte. To better understand its mechanism of action, we have also investigated IL-12 signal transduction and compared it to IL-2 signaling. We demonstrated that it activated JAK2 and TYK2 Janus kinases and activated the STAT4 transcription factor.

It is hoped that understanding the precise mechanisms involved in cytokine signaling will provide avenues for the development novel agents that can regulate the immune response in a therapeutically desirable manner.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 AR 41107-01 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Implementation Errors In Functional Movement Analysis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
MC Brown IRTA Fellow, Biomechanics Laboratory ARB/NIAMS

COOPERATING UNITS (if any)

Rehabilitation Medicine Department/CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.85

PROFESSIONAL:

0.85

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Instrumentation, implementation, and tracking errors occur during functional movement analysis. Instrumentation errors have been adequately addressed by other investigators. Implementation errors continue to be difficult to identify. Implementation and tracking errors are commonly associated with discontinuities (shifts and spikes) in target trajectory time history data. Shifts occur when the combination of cameras used to locate a target changes. Spikes occur when target images merge with other target images or with camera strobes. The purpose of this study was to develop a technique for identifying discontinuities, in order to prevent implementation errors and tracking errors from becoming errors in clinical judgement.

To date, data from three walking trials were evaluated. Software was used to identify discontinuities in the data by calculating discontinuity parameters (DPs). The DP was calculated by scanning data for sample to sample changes in target translation accelerations. Each target trajectory discontinuity associated with a joint angle error larger than 20% of the normal joint range of motion (ankle > 8 degrees, knee > 12 degrees, hip > 8 degrees in the sagittal plane) was associated with a DP larger than 20m/s². There was no obvious correlation between the magnitude of the DP and the size the joint angle error associated with the discontinuity. The largest DP measured 42m/s² and was associated with a joint angle error of 13 degrees at the knee and 22 degrees at the hip in the leg model that was used for testing.

It was determined that discontinuity parameter calculation is an excellent technique for identifying discontinuities in target trajectory data. The results of this study were presented at the North American Clinical Gait Laboratories Conference in Waterloo, Ontario, and an abstract was published in Gait and Posture during the month of June, 1995.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41108-01 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Joint Moments In Functional Movement Analysis and Isokinetic Testing

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
MC Brown IRTA Fellow, Biomechanics Laboratory ARB/NIAMS
CS Graff Summer Biologist ARB/NIAMS

COOPERATING UNITS (if any)

Rehabilitation Medicine Department / CC

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

0.34

PROFESSIONAL:

0.17

OTHER:

0.17

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to determine the relationship between joint moments in functional movement and joint moments in isokinetic testing. Isokinetic testing will be performed in order to quantify the maximal ankle, knee, and hip moments that subjects can create under isokinetic (open kinetic chain) conditions. Kinematic and kinetic data will be collected using a functional movement analysis system, while subjects ascend a stair while carrying increasing loads. The ankle, knee, and hip moments required to perform this functional (closed kinetic chain) task with maximum loads, will be compared to the maximum moments created during isokinetic testing. Clarification of the relationship between the joint moments required for functional movement and the joint moments measured during isokinetic testing may allow for predictions of functional capacity based on isokinetic strength testing.

To date, two subjects have been evaluated. Kinetic data during stair ascent trials revealed an increase in the joint moments generated at the ankle, knee, and hip as loads were increased from no weight to 50% body weight.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41109-01 ARB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pathogenesis and Natural History of Psoriatic Arthritis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

P.I.:	D.T. Boumpas	Visiting Scientist	ARB, NIAMS
Others:	I.O. Tassiulas	Visiting Fellow	ARB, NIAMS
	C. Yarboro	Research Nurse	ARB, NIAMS
	J.J. DiGiovanna	Senior Investigator	ARB, NIAMS
	J.H. Klippel	Senior Investigator	ARB, NIAMS
	D.L. Kastner	Senior Investigator	ARB, NIAMS

COOPERATING UNITS (if any)

None

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892-1828

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0.1

CHECK APPROPRIATE BOX (ES)

☒ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Patients with known or suspected psoriatic arthritis are evaluated at the Clinical Center. Studies include characterization of the clinical and laboratory features of the disease, research studies of the natural history and pathogenesis, genetic studies, and determination of the patients eligibility to enter experimental therapeutic protocols. Patients are asked to contribute blood, skin or synovial membrane samples for immunologic and genetic research studies. Immunologic studies include investigations of 1) the cytokine profile of the disease (type 1 vs type 2 immune response); 2) the role of the interaction of T cells with antigen presenting cells; and 3) the regulation of apoptosis of keratinocytes and synoviocytes. Genetic studies include establishment of pedigrees with multiplex affected families and genetic linkage analysis.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41110-01 ARB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Newer Adenosine Analogs as Immunosuppressive Agents

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

P.I.:	D.T. Boumpas	Visiting Scientist	ARB, NIAMS
Others:	J. Davis	Clinical Associate	ARB, NIAMS
	I.O. Tassiulas	Visiting Fellow	ARB, NIAMS
	C. Yarboro	Research Nurse	ARB, NIAMS
	J.H. Klippel	Senior Investigator	ARB, NIAMS

COOPERATING UNITS (if any)

Clinical Center Pathology, Immunology Section (Dr. Thomas Fleisher)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892-1828

TOTAL STAFF YEARS:

1.1

PROFESSIONAL:

1.0

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The clinical efficacy, toxicity, and immunological effects of the newer adenosine analogs, such as fludarabine are being studied in patients with autoimmune rheumatic disorders in phase I/II clinical studies. These agents primarily affect lymphoid cells because these cells contain an enzyme necessary for activation of these compounds. Previous investigations employing these drugs in patients with low grade lymphoid malignancies have shown that they selectively target immune cells by inducing a physiologic form of cell death called apoptosis. Their effects on the clinical course of immunologic diseases, as well as on the function of immune cells have not been characterized.

Twelve patients have been entered into a study for treatment of refractory rheumatoid arthritis and one into a study for the treatment of psoriatic arthritis. To date, no major or unexpected toxicities have been encountered. Fludarabine treatment has resulted in sustained B and T lymphopenia (B>T). We are attempting to determine whether the level of lymphopenia should be a major determinant of safe drug doses; no substantive changes in total immunoglobulin or antibody levels have been seen. At the low doses of fludarabine used, significant clinical effects have been observed in eight patients with rheumatoid arthritis who have completed this therapy so far.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 AR 41111-01 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical and Therapeutic Studies of the Rheumatic Diseases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,

PI: H. Ralph Schumacher IPA, U. Pennsylvania

J. Pando Medical Staff Fellow, ARB

C. Yarboro Research Nurse, ARB

M. Gourley Senior Staff Fellow, ARB

V. Gluck Pre-Doctoral IRTA, ARB

R. Wilder Senior Investigator, ARB

J. H. Klippel Clinical Director, NIAMS

K. Kanik Medical Staff Fellow, ARB

COOPERATING UNITS (if any)

LAB/BRANCH

Arthritis and Rheumatism Branch

SECTION

Clinical Investigations Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study proposes to establish a cohort of patients with inflammatory arthritis of less than one year's duration in an attempt to better understand pathogenetic mechanisms involved in early synovitis and to determine prognostic factors associated with the clinical disease course. Synovial biopsies and synovial fluid will be obtained to search for microbial agents and other initiating and modulating factors that may be most readily distinguished early in the disease and to determine the stage of disease at which certain immunologic and hormonal changes become evident. Specimens will be evaluated by polymerase chain reaction and immunoelectron microscopy for the presence of infectious agents such as difficult to culture chlamydia and to assess cytokine profiles present with the synovial membrane. The study will search for genetic, in particular HLA DR loci, and other features that may be associated with specific forms of inflammatory arthropathies that might predict the subsequent clinical disease course or response to different agents used in treatment of rheumatoid arthritis and other types of chronic inflammatory arthropathies. Assessments of the neuroendocrine axis suggest that low cortisol or androgens favor development of rheumatoid arthritis. These studies will contribute to knowledge about the early pathogenic events in early arthritis and have the potential to assist in development of new approaches to patient management.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01-AR-41112-01 ARB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Treatment & Pathogenesis of Skin Cancer, Disorders of Cornification & Psoriasis

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
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INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

2.5

2.5

0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The goal of these studies is to explore the efficacy, toxicity, and mechanisms of action of new treatments, and to better understand the pathogenesis of dermatologic diseases, particularly skin cancer and the disorders of cornification. Patients followed long-term at NIH have established the efficacy and characterized the toxicity of isotretinoin and etretinate for several skin disorders. These patients continue to be monitored to characterize skeletal toxicity, an important chronic side effect. Understanding the scope of chronic retinoid toxicity is important because of the expanding number of synthetic retinoids under clinical study. The peripheral skeletal involvement that frequently occurs after chronic etretinate therapy was first identified in this cohort. We have now discovered that osteoporosis is also a toxicity of etretinate. In patients with Darier's disease, we have identified a novel, common, cystic bone abnormality. Studies directed at skin cancer treatment and prevention are continuing. We demonstrated the effectiveness of oral isotretinoin as a chemopreventive agent in patients with high rates of skin cancer formation. These patients are now maintained on long-term isotretinoin chemoprevention to determine optimum dosing. A recently completed phase I/II study of gamma interferon for basal cell carcinoma demonstrated shrinking of all tumors, but few completely regressed. We have begun a phase I/II study of topical antiflammin, a novel peptide inhibitor of phospholipase A2, which has homology to uteroglobin and lipocortin. Our collaboration with the Lab. of Skin Biology has expanded (Z01-AR-41089-04 LSB). We were the first to find linkage of the epidermolytic hyperkeratosis gene to the type II keratin gene cluster on chromosome 12q, and have now identified mutations in keratin 1 or 10 in 12 families. Based on our clinical studies we have created a new classification of the disease including 6 distinct clinical phenotypes. Correlation of mutations with clinical phenotypes will lead to a better understanding of the relationship between keratin structure and function. In a pedigree with a non-epidermolytic form of palmar/plantar keratoderma (Unna-Thost disease) we found complete linkage with the type II keratin region and discovered a mutation in the V1 domain of the keratin 1 gene as the cause of this disease. Our fine mapping study of Darier's disease narrowed the location of the disease gene to a 5 cM interval on chromosome 12q23-q24.1. In lamellar ichthyosis we were first to find linkage to chromosome 14q11 and have now identified mutations in transglutaminase 1 as the cause of the disease. Detailed clinical analysis will allow correlation of mutations with clinical phenotypes.

ZO1AR41025-24 ARB

Studies of the Cell Surface Receptor for IgE

Metzger, H

The high affinity receptor for IgE on mast cells & basophils (Fc(epsilon)RI) plays a central role in immediate hypersensitivity reactions. Reaction of receptor-bound IgE with polyvalent antigen clusters the receptors & this stimulates cellular secretion of both preformed & newly synthesized mediators of inflammation. Our studies focus on the molecular mechanisms by which aggregation of the receptors generate these cellular responses. The similarity of Fc(epsilon)RI to other receptors of the immune system (e.g. the clonotypic receptors on T & B lymphocytes), make it likely that the significance of such studies extends beyond the IgE/mast cell system. During the past year, our principal progress was in two areas: 1) Completing the first phase of our studies on the association between a tyrosine kinase and the receptor. Using either gentle isolation procedures or chemical crosslinking, we found convincing evidence for the following mechanism by which the topological change induced by aggregation of the receptors is converted into a biochemical signal: a) Some unaggregated receptors are constitutively associated with the src-like tyrosine kinase--p55/56lyn in the case of receptors in the rat "RBL" cell-line with which we work. b) Aggregation of receptors in effect induces the formation of enzyme-substrate complexes so that the kinase on one receptor can (trans)phosphorylate other receptors newly approximated to it. c) Such phosphorylated receptors "recruit" additional kinase thereby initiating the biochemical cascade. 2) Further analysis on the dynamics of signal transduction in relation to aggregation of the receptors in vivo. Quantitative analysis of the relationship of aggregation to phosphorylation of receptor tyrosines revealed a discrepancy that cannot be accommodated by any simple model. The data suggest that not only the amount but also the rate of aggregation may influence the cellular response. These results have general implications on the role of ligand-receptor interactions in initiating alternative cellular responses.

ZO1AR41048-16 ARB

Bacterial Cell Wall-Induced Arthritis in the Rat

Wilder, RL

We and others have shown that Lewis (Lew) rats are highly susceptible to a wide spectrum of autoimmune diseases such as bacterial cell wall arthritis. We have also demonstrated that they develop severe inflammation in response to nonspecific proinflammatory agents such as carrageenin. In contrast, Fischer (F344) rats develop minimal or no inflammation in response to the same stimuli. We have previously provided data suggesting that these differences are related to a global deficiency in hypothalamic-pituitary-adrenal axis (HPA) responsiveness in Lewis rats. We have continued to characterize the functional basis of the HPA axis defect and have now shown that Lewis and Fischer rats respond similarly to corticosteroids. These data render unlikely the hypothesis that the HPA axis defect in Lewis rats is a consequence of primary abnormalities in type 1 corticosteroid receptors or tissue sensitivity to corticosteroids.

We have also continued to characterize cytokine responses of Lewis and Fischer rats following LPS injection and have noted several prominent differences in these strains. These responses parallel the HPA differences. As an extension of these studies, we have also begun to clone

genes that are differentially expressed in these strains in response to LPS. To date, we have cloned 2 novel transcripts (LF-1 and LF-2) and are sequencing these mRNAs. These data may provide important insights into the mechanisms regulating susceptibility and resistance to autoimmune inflammatory diseases.

Z01AR41066-13 ARB

Characterization of synovial tissues from patients with RA and related conditions
Wilder, RL

Previous studies from our laboratory have suggested that angiogenesis plays a major role in rheumatoid arthritis (RA). Related studies from our laboratory have indicated that enhanced expression of cyclooxygenase-2 (Cox-2) is also important and parallels the intensity of the inflammatory process and angiogenesis. We have now defined a potential biochemical mechanism that may link these observations. Interleukin-1 (IL-1), which is a powerful stimulator of Cox-2, and prostaglandin E2, which is product of Cox-2, induce the expression of vascular endothelial growth factor (VEGF) in rheumatoid synovial fibroblasts. VEGF expression is upregulated in rheumatoid synovium and probably plays a role in driving angiogenesis. These data may also provide insights into mechanisms by which corticosteroids or nonsteroidal inflammatory drugs regulate inflammatory angiogenesis. These agents have powerful suppressive effects on Cox-2 and prostaglandin E2 production. In addition, corticosteroids suppress interleukin-1 production.

Our laboratory is also characterizing cytokine transcription in synovial tissues from patients with early synovitis. Subjects include those with rheumatoid arthritis, reactive arthritis, a various other forms of arthritis. We have noted that the expression of tumor necrosis factor-alpha (TNF-alpha) and IL-1 in virtually all forms of early synovitis. Similarly, gamma interferon expression is common, whereas IL-4 and IL-13 expression is rare. These data suggest that macrophage-derived and type 1 lymphocyte-derived cytokines, in contrast to type 2 cytokines, predominate in early synovitis, regardless of the type of disease. The data suggest the cell-mediated, rather than humoral mechanisms play essential roles in the early stages of the inflammatory process in the joints.

Z01AR41074-08 ARB

Studies on etiology and pathogenesis of myositis
Plotz, PH

Idiopathic inflammatory myopathy (polymyositis, dermatomyositis, and related disorders) is a family of inflammatory diseases in which disease-specific autoantibodies occur and for which there is considerable indirect evidence pointing to a viral etiology. We have over the past several years, seen and studied and collected serum, blood, and muscle specimens from well over 500 patients suspected of having myositis and we have collected epidemiologic information on many patients. Our recent attention has been focussed on the mechanism of autoimmunity and on the details of tissue within the affected muscles.

We have completed studies on the primary sequence of two more of the target autoantigens, isoleucyl- and (in collaboration with Paul Schimmel at MIT) alanyl-tRNA synthetase and have identified the chromosomal location of four of the target aminoacyl-tRNA synthetases. We have produced pure recombinant histidyl-tRNA synthetase (HRS) and, with Craig Hyde, made large crystals, but the x-ray diffraction studies have so far been of only low resolution. Dr. Terry O'Hanlon and Dr. Fred Miller of CBER/FDA with Dr. Raben, have identified a transcript of what appears to be the mitochondrial HRS.

We have studied the cytokines and chemokines produced by circulating leukocytes (with Dr. Dennis Klinman of CBER/FDA) and by cells within affected muscle biopsies, and we have continued preparations to study the peptides found in Class I HLA antigens on the surface of myoblasts.

ZO1AR41076-08 ARB

Therapeutic trials in idiopathic inflammatory myopathies
Plotz, PH

In an attempt to find better ways to treat myositis, the group has explored the use of both established medications used in new ways or combinations or new therapies. These trials are virtually the only such trials of the treatment of this very uncommon condition and thus also serve to draw patients to the Clinical Center for the other research projects carried out by the group on the basic mechanisms of myositis and related or mimicking diseases (outlined in ZO1 AR 41074-08 and ZO1 AR 41099-03).

A randomized, crossover trial of high dose intravenous methotrexate with leucovorin rescue and of a combination of oral methotrexate and azathioprine has been completed in the past year and an analysis of the results is being prepared for publication.

We have begun a trial of the purine analogue, fludarabine, in the therapy of myositis. A quantitative method of assessing muscle inflammation in myositis has been developed and will be used in current and future therapeutic trials. Thirteen of the planned sixteen patients have entered the trial so far, of whom almost half have completed the six-month treatment phase.

A trial of the anti-thyroid drug, methimazole, will be begun in the next year. This agent was chosen because it may alter the ability of muscle cells to serve as a target for immunologic attack. In this past year, the methods for assessing the effect of the drug on muscle and blood cells have been developed in collaboration with NIDDK & NCI.

ZO1AR41083-06 ARB

Genetics of Familial Mediterranean Fever
Kastner, DL

Familial Mediterranean fever (FMF) is characterized by episodes of fever with abdominal pain, pleurisy, and/or arthritis; some patients also develop systemic amyloidosis, leading eventually to renal failure. FMF is inherited as a single autosomal recessive gene (designated MEF), which is

very common among Jewish, Armenian, Turkish, and Arab populations. Since the biochemical lesion in FMF is unknown, our goal is to identify the FMF gene by positional cloning. By the beginning of this year, we had narrowed the candidate interval to a region of approximately 1 Mb on the short arm of chromosome 16. In 1994-95 our efforts were divided among a) genomic cloning of the candidate interval; a) further genetic refinement of the interval; c) transcript identification; and d) functional studies of FMF leukocytes.

Genomic cloning began with the identification of CEPH megaYACs. However, due to the instability of megaYACs from this part of the genome, a major effort in the last year has been directed towards developing a cosmid contig. Using the Los Alamos 10X chromosome 16-specific cosmid library, we have constructed a 525 kb contig on the telomeric end of the 1 Mb candidate interval, and a 350 kb contig on the centromeric end. We have made NotI and EcoRI restriction maps of these contigs. We are attempting to close the gap between the two contigs with a combination of cosmids, YACs, PACs, BACs, and P1s.

Refined genetic mapping was facilitated by our identification of 4 new highly polymorphic microsatellites from the cloned genomic DNA. These new markers exclude an additional 250 kb on the telomeric end of the interval, and 300 kb on the centromeric end of the interval. Moreover, at one of the 4 marker loci there are no recombinants among a panel of 65 Jewish, Armenian, and Arab families. A specific allele of this latter locus is in strong linkage disequilibrium with FMF among North African Jewish FMF patients; Luria-Delbruck analysis of linkage disequilibrium data in the Moroccan Jewish founder population places MEF within about 80 kb of this marker.

We are identifying transcripts from the FMF candidate interval by exon amplification from cosmids. To date, we have isolated a total of 28 putative exons from the region, and we are screening cDNA libraries for the corresponding full-length transcripts. In parallel, collaborators in Australia are using direct cDNA selection on cosmid pools to identify additional transcribed sequences.

Phenotypic analysis of FMF leukocytes may facilitate analysis of positionally-defined candidate genes; over the last year we have confirmed that leukocytes from FMF patients show increased adhesion to fibronectin and type I collagen, but express normal levels of known cell-surface adhesion molecules.

Z01AR41088-05 ARB

Genetic and Neuroendocrine Factors in the Autoimmune Diseases

Wilder, RL

Studies from animal models have suggested the possibility that hypothalamic-pituitary-adrenal axis (HPA) and other neuroendocrine dysfunctions may play a role in rheumatoid arthritis (RA) and other autoimmune conditions. For these and other reasons, we are conducting studies investigating neuroendocrine function in patients with RA and related arthropathies. We have completed one study that supports our view that adrenal hormone production is blunted in patients with RA. We investigated circadian secretion of ACTH, cortisol, interleukin-1 (IL-1)

and IL-6 and demonstrated that RA patients show "normal" or occasionally low 24-hour cortisol production, while plasma IL-6 levels are markedly elevated. Of interest, we noted that IL-6, like ACTH and cortisol, is secreted with a circadian pattern, and the pulses of cortisol are linked to pulses of IL-6, with IL-6 leading cortisol by about 1 hour. Linkage of IL-6 secretion to ACTH secretion was much weaker. These data suggest that IL-6 may directly stimulate adrenal secretion of cortisol, but, more importantly, the data support our hypothesis that adrenal cortisol production in RA patients is "inappropriately normal" or blunted. We would expect markedly increased cortisol production the setting of high IL-6 levels. Additional data have been collected on adrenal androgen levels in new-onset RA patients. Interestingly, the production of adrenal androgens, such as dehydroepiandrosterone (DHEA) and (DHEA-S), is even more abnormal than cortisol. These data indicate that adrenal hypofunction in RA patients is not restricted to cortisol but suggest more global neuroendocrine dysfunction.

Initial studies of HPA axis and sympatho-adrenal function in patients with fibromyalgia were completed. These patients exhibit blunted HPA responses to corticotropin releasing hormone (CRH) and strikingly low levels of neuropeptide Y. These data suggest abnormalities in the two major components of the stress response system may play a role in this perplexing condition.

We also completed a study testing the hypothesis that corticosteroids mediate some of their peripheral antiinflammatory effects by stimulating somatostatin, a well-known antiinflammatory peptide. In an animal model of aseptic inflammation, we noted increased local expression of somatostatin following corticosteroid treatment in concert with suppression of local proinflammatory substances such as substance P, CRH and tumor necrosis factor-alpha.

Z01AR41092-04 ARB

Mapping of Genes and Genetic Polymorphisms in Rats
Wilder, RL

Rats are an important experimental model for many human diseases, many of which have a genetic component. As another approach to investigating fundamental origins of autoimmune diseases, we have initiated studies with the ultimate goal of mapping genes regulating autoimmune disease in rats. A initial and critical step to meet this objective is the development of a genetic linkage map applicable to autoimmune-disease prone and -resistant rat strains. Over the past year, we have identified more than 300 new polymorphic DNA markers that distinguish various inbred rat strains (total of about 900 since the initiation of the project). We have now mapped a large fraction of these markers in F2 progeny of LEW x F344 (more than 400 markers total), LEW x BN (more than 400 markers), or DA x F344 (about 200 markers).

The genetic map that we have developed has been used to refine our previous localization of the rat athymic nude trait on rat chromosome 10. We have also now refined our localization of the rat osteopetrosis gene (op) to the distal end of rat chromosome 10.

Several efforts are in progress to map phenotypes related to autoimmune disease in F2 progeny of LEW X F344 rats. In addition, we are mapping genes that regulate collagen-induced arthritis (CIA), an autoimmune disease model that resembles rheumatoid arthritis, in F2 progeny of DA x

F344 rats. Over the past year, we have generated data in the LEW X F344 progeny indicating that virtually all of the phenotypes under investigation are subject to complex regulation involving 2-4 genes in distinct chromosomal locations. In the CIA model, we have identified a major regulatory locus (LOD approximately 63), linked to the major histocompatibility complex (MHC) on chromosome 20. This locus acts dominantly and contributes approximately 50% to the phenotypic variability. In addition, at least 4, and possibly 5, additional chromosomal regions (chromosomes 1,4,7 and 14) contribute to phenotypic variability. The mode of action of the non-MHC genes is still being analyzed.

Z01AR41095-04 ARB

Translational and rotational power terms in 6 degree-of -freedom joint modeling
Holden, JP

This project was inactive during the period covered. Separate but related projects were undertaken to better understand the source(s) of the joint translations previously measured at the ankle and knee.

Z01AR41096-04 ARB

Nonequilibrium in the translation equations of motion
Holden, JP

This project was inactive during the period covered.

Z01AR41099-04 ARB

The genetic basis for metabolic myopathies
Raben, N

In the course of studying autoimmune mechanisms in myositis, an inflammatory muscle disease, a number of patients with other muscle disease have been evaluated. Two genetic metabolic myopathies, phosphofructokinase (PFK) deficiency and acid maltase (GAA) deficiencies, have been selected for studies at the molecular level.

The studies of PFK deficiency, now drawing to a close, have focussed on defining the genetic abnormalities in different patient populations, and a number of mutations have been found. Experiments in collaboration with a laboratory in Germany have used a yeast expression system to study the biochemical properties of the abnormal enzymes.

Studies of GAA deficiency have centered on the adult form of the disease. An infantile form is fatal in the first year of life. Studies on the mechanism of a common GAA mutant genotype, shared by 70% of adult-onset patients, have been expanded in a model system. This system enabled us to identify a transcriptional silencer in the GAA gene, which has implications for gene therapy. Experiments to lay the ground work for therapeutic gene transfer with a recombinant retrovirus bearing the normal cDNA resulted in the restoration of GAA activity in affected fibroblasts. Similar experiments with affected myoblasts are ongoing. Plans are underway for developing a GAA deficient mouse model for in vivo gene therapy.

Z01AR41100-03 ARB

Skeletal tracking with skeletal fixation

Holden, JP

Quantitative gait analysis requires attachment of optical targets to the skin's surface. Data derived from monitoring the locations of these targets while a patient walks provide the basis for an objective study of the movement. The movement of the skin and underlying soft tissues relative to the bone is known to be a source of error in the measurements, but the magnitude of the errors is unknown and goes uncorrected in standard analyses. The purpose of this study is to evaluate the magnitude and pattern of error produced by tracking the skeletal system using surface mounted targets.

As a subject walks in the laboratory, a video-based target tracking system is used to measure simultaneously the movement of two sets of reflective targets attached to each body segment being studied -- one set mounted to the surface (standard technique) and one mounted to the bone. Skeletal fixation of the bone-mounted targets is accomplished using a percutaneous skeletal tracker (PST). Each target set is used separately in calculations of segment motions (kinematics) and the net joint forces and moments (inverse dynamics analysis) that cause the motions. The differences in results represent the errors introduced by the surface target attachment technique. Inverse dynamics analyses were performed on data from three subjects who walked with the PST attached to the shank (lower leg). The magnitudes of the differences in forces and moments would not likely affect the clinical interpretation of the data, but may be important for other applications such as musculoskeletal modeling. Additional data were also collected to measure the motion with respect to bone of a single surface mounted target on the lateral aspect of the knee. The motion of this target (used by some laboratories to estimate the location of the knee joint center) was compared with the joint center estimate derived from the set of surface mounted targets at the mid-shank. The movement of the single lateral knee target with respect to bone during gait was more than twice that of the knee joint center estimated from the surface mounted targets.

The PST is currently undergoing modification and cadaveric testing to establish a design and attachment technique for use on the femur.

Z01AR41101-02 ARB

Intracellular Signaling by the Cell Surface Receptor for IgE

Rivera, J

The molecular mechanisms that lead to the expression of newly synthesized mediators of inflammation are the central focus of our studies. We utilize a mast cell model, RBL-2H3 cells, due to the robust production of both inflammatory and immunoregulatory cytokines and the induction of their synthesis via the well characterized high affinity receptor for IgE (FcEpsilonRI). In particular the steps involved in the signaling pathway, beginning with IgE-antigen activation of the high affinity receptor for IgE and resulting in the induction of cytokine genes, are being explored. In the past year we have focused principally on the regulation of kinases, receptor adapter molecules and transcription factors known to be critical for the

induction of gene expression. Three principal areas of investigation are underway: 1) Studies on the regulation of molecules purported to link cell surface activation of receptors with induction of gene expression. 2) Exploring the regulation of receptor-associated protein kinase C (PKC) delta in response to aggregation of the high affinity receptor for IgE. 3) Development of stably transfected mast cell lines over-expressing the individual isozymes of PKC to enable studies on cellular targets of enzyme activity.

Our new results show: 1) The calcium independent PKC-delta is tyrosine phosphorylated in response to receptor aggregation. This tyrosine phosphorylation of PKC delta inhibits its ability to recognize the receptor gamma chain as a substrate, but does not inactivate the enzyme since it readily phosphorylates other substrates. This result suggests a novel intracellular mechanism for regulation of substrate recognition. 2) In collaborative studies we have identified the site of phosphorylation as tyrosine 52 in the regulatory domain of PKC delta. 3) Studies on the regulation of Vav, a protein thought to play a role in MAP kinase activation and therefore gene transcription, show that this protein which we have found to associate with the FcEpsilonRI exists as a multicomponent complex in association with the adaptor molecule Grb2, the serine/threonine kinase Raf-1 and the p42 MAP Kinase. Evidence is provided of a link between receptor and MAP kinase activation.

Z01AR41102-03 ARB

Genetics of Cystinuria

Kastner, DL

Cystinuria is an autosomal recessive disease in which there is excessive excretion of cystine in the urine, with precipitation of urinary cystine stones. Patients with this disorder may have renal colic, urinary tract obstruction, secondary urinary tract infection, or, if untreated, renal insufficiency. By the beginning of this reporting period we had mapped a cystinuria susceptibility gene to the short arm of chromosome 2, and had identified mutations in the SLC3A1 gene (which is located in the appropriate chromosomal region) in one American cystinuria family. Another group had independently identified 6 other SLC3A1 mutations in cystinuria. During the last year our goals have been to screen our panel of 17 cystinuria families for additional mutations, and to elucidate the genomic structure of SLC3A1.

Including the 2 mutations reported last year, we have now identified a total of 5 cystinuria-associated SLC3A1 mutations. This includes: a) a frameshift mutation and b) a large deletion in a non-Jewish American family; c) a C-to-T nonsense mutation at cDNA position 808 in 1 Israeli Druze and 4 Ashkenazi Jewish families (this mutation was found in 8 of 8 Ashkenazi carrier chromosomes examined); d) a C-to- A transversion at cDNA position 383 in a Persian and a Yemenite Jewish family; and e) an A-to-G transition in the splice donor site of exon 4, causing skipping of this exon, in a Moslem Arab family.

Studies of the genomic structure of SLC3A1 were based on our identification a P1/PAC contig from the appropriate chromosomal region. SLC3A1 contains 10 exons, and spans a total of approximately 45 kb of genomic DNA. We have constructed an EcoRI restriction map of the region, and have developed PCR primers from introns that allow exon amplification from

genomic DNA, thus obviating the need for RT-PCR on illegitimate transcripts from lymphoid cells. We have also sequenced 650 bp upstream from the initiation codon, and have identified several regulatory elements in the promoter region.

Finally, we have obtained evidence that, in several Libyan Jewish families, the cystinuria susceptibility gene is not SLC3A1. This is based on the following observations on these families: a) for 5/5 individuals tested (3 of them from inbred families), we were unable to find mutations in the SLC3A1 cDNA; b) by urine amino acid analysis, we have confirmed the affection status of all individuals; c) using an intragenic microsatellite that we identified, we have found definite SLC3A1 recombinants; and d) haplotype analysis with other newly identified flanking genetic markers is also incompatible with SLC3A1 causing cystinuria in these families. Future studies will be directed toward the mapping and eventual cloning of a second cystinuria susceptibility gene.

Z01AR41103-03 ARB
Genetics of Rheumatoid Arthritis
Kastner, DL

This project was inactive during the reporting period.

Z01AR41104-03 ARB
Clinical and Therapeutic Studies of the Rheumatic Diseases
Klippel, JH

We are conducting a series of clinical investigations involving patients with systemic lupus erythematosus in which questions related to disease pathogenesis, epidemiology, therapy and comorbidities are addressed. Therapeutic studies in progress include a long-term, randomized trial of corticosteroids and immunosuppressive drugs in patients with severe, proliferative lupus glomerulonephritis and a phase I/II of the nucleoside analog 2-chlorodeoxyadenosine similarly in patients with lupus nephritis. Studies are underway to assess risk factors associated with the development of osteoporosis and vascular thrombotic events. Collaborative studies with scientists interested in lupus pathogenesis during the past year have included the influences of cytokines in autoimmunity (D. Klinman), antibody binding to chromatin adherent to the collagen of the glomerular basement membrane (JB Lefkowitz), and FcγRIIIa alleles as risk factors for nephritis in African-Americans (JE Salmon).

Z01AR41105-02 ARB
The Effect of Walking Speed on the Mechanics of Gait
Holden, JP

Neuromuscular function and adaptation are often evaluated by comparing a patient's kinematic (movement) and kinetic (forces and moments) gait patterns with those obtained from healthy individuals. A feature of many patients' gait is a much slower than normal walking speed. It is important when interpreting a patient's data to distinguish which variations from normal are due solely to differences in walking speed and which are due to neuromuscular abnormalities or

adaptations other than speed. The purpose of this project is to investigate the effect of dramatically different walking speeds on the mechanics of gait. One specific objective is to determine if a "quadriceps avoidance" pattern of net knee flexion-extension moments (reported in the literature for patients with anterior cruciate ligament deficiency) is demonstrated by healthy subjects at slower walking speeds.

Movement and force data have been collected from 18 healthy subjects (9 male, 9 female, ages 21-37 yrs) who walked at 25%, 50%, 75%, 100%, and 125% of a normalized speed of 0.785 statures/s ($\pm 2.5\%$). Speed was measured over a 2.28 m distance using two photocells and a gait speed indicator circuit. Inverse dynamics analysis was performed to calculate the net flexion-extension moments at the knee during the stance phase of gait.

Two-thirds of individual subjects had knee flexion-extension moment patterns that were similar across all five walking speeds. One-third of the subjects, however, demonstrated a quadriceps avoidance strategy (net flexor moment or zero moment) in at least one trial at the slowest speed. Decreased moment amplitude was related to decreased knee flexion during the "loading response" phase in early to mid stance. The subjects who produced the quadriceps avoidance pattern at the slowest speed did so by keeping the knee near full extension throughout the stance phase of gait.

ZO1AR41106-01 ARB

Biochemistry of Lymphocyte Activation

O'Shea, JJ

Interleukin 2 and other cytokines dramatically influence the immune response and therefore understanding the molecular basis of cytokine signal transduction should provide important insights into lymphocyte activation. To this end, we cloned a new protein tyrosine kinase, termed JAK3 and showed that it is a critical element in signalling via the subfamily of cytokine (IL-2, IL-4, IL-7, IL-9, and IL-15) receptors that use the common γ chain (γ c). We further showed that whereas JAK3 binds γ c, JAK1 binds the IL-2R β . X-linked severe combined immunodeficiency (X-SCID) is due to mutations of γ c. We demonstrated that several γ c mutations result in the failure of this subunit to bind JAK3, whereas reconstitution of cell lines with γ c mutations with the wild type allele restores the ability to activate JAK3 and JAK1. The disease X-linked combined immunodeficiency (X-CID) is also due to a mutation of γ c. This allele binds JAK3 but does so at much reduced efficiency and this binding correlates with the reduced severity of this immunodeficiency. In addition, we have also now identified patients with SCID who lack JAK3 indicating that JAK3 and its function appear to be critical in the development of the immune system.

We have also investigated cytokine activated transcription factors, in particular STAT protein which are tyrosine phosphorylated transcription factors that contain SH2 domains. We have shown that IL-2 activates STAT3 and STAT5. IL-12 is a cytokine that has profound effects on T lymphocyte. To better understand its mechanism of action, we have also investigated IL-12 signal transduction and compared it to IL-2 signaling. We demonstrated that it activated JAK2 and TYK2 Janus kinases and activated the STAT4 transcription factor. It is hoped that understanding

the precise mechanisms involved in cytokine signaling will provide avenues for the development of novel agents that can regulate the immune response in a therapeutically desirable manner.

ZO1AR41107-01 ARB

Implementation Errors In Functional Movement Analysis

Brown, MC

Instrumentation, implementation, and tracking errors occur during functional movement analysis. Instrumentation errors have been adequately addressed by other investigators. Implementation errors continue to be difficult to identify. Implementation and tracking errors are commonly associated with discontinuities (shifts and spikes) in target trajectory time history data. Shifts occur when the combination of cameras used to locate a target changes. Spikes occur when target images merge with other target images or with camera strobes. The purpose of this study was to develop a technique for identifying discontinuities, in order to prevent implementation errors and tracking errors from becoming errors in clinical judgement.

To date, data from three walking trials were evaluated. Software was used to identify discontinuities in the data by calculating discontinuity parameters (DPs). The DP was calculated by scanning data for sample to sample changes in target translation accelerations. Each target trajectory discontinuity associated with a joint angle error larger than 20% of the normal joint range of motion (ankle > 8 degrees, knee > 12 degrees, hip > 8 degrees in the sagittal plane) was associated with a DP larger than 20m/s². There was no obvious correlation between the magnitude of the DP and the size of the joint angle error associated with the discontinuity. The largest DP measured 42m/s² and was associated with a joint angle error of 13 degrees at the knee and 22 degrees at the hip in the leg model that was used for testing.

It was determined that discontinuity parameter calculation is an excellent technique for identifying discontinuities in target trajectory data. The results of this study were presented at the North American Clinical Gait Laboratories Conference in Waterloo, Ontario, and an abstract was published in *Gait and Posture* during the month of June, 1995.

ZO1AR41108-01 ARB

Joint Moments In Functional Movement Analysis and Isokinetic Testing

Brown, MC

The purpose of this study is to determine the relationship between joint moments in functional movement and joint moments in isokinetic testing. Isokinetic testing will be performed in order to quantify the maximal ankle, knee, and hip moments that subjects can create under isokinetic (open kinetic chain) conditions. Kinematic and kinetic data will be collected using a functional movement analysis system, while subjects ascend a stair while carrying increasing loads. The ankle, knee, and hip moments required to perform this functional (closed kinetic chain) task with maximum loads, will be compared to the maximum moments created during isokinetic testing. Clarification of the relationship between the joint moments required for functional movement and the joint moments measured during isokinetic testing may allow for predictions of functional capacity based on isokinetic strength testing.

To date, two subjects have been evaluated. Kinetic data during stair ascent trials revealed an increase in the joint moments generated at the ankle, knee, and hip as loads were increased from no weight to 50% body weight.

ZO1AR41109-01 ARB

Pathogenesis and Natural History of Psoriatic Arthritis

Boumpas, DT

Patients with known or suspected psoriatic arthritis are evaluated at the Clinical Center. Studies include characterization of the clinical and laboratory features of the disease, research studies of the natural history and pathogenesis, genetic studies, and determination of the patients eligibility to enter experimental therapeutic protocols. Patients are asked to contribute blood, skin or synovial membrane samples for immunologic and genetic research studies. Immunologic studies include investigations of 1) the cytokine profile of the disease (type 1 vs type 2 immune response); 2) the role of the interaction of T cells with antigen presenting cells; and 3) the regulation of apoptosis of keratinocytes and synoviocytes. Genetic studies include establishment of pedigrees with multiplex affected families and genetic linkage analysis.

ZO1AR41110-01 ARB

The Newer Adenosine Analogs as Immunosuppressive Agents

Boumpas, DT

The clinical efficacy, toxicity, and immunological effects of the newer adenosine analogs, such as fludarabine are being studied in patients with autoimmune rheumatic disorders in phase I/II clinical studies. These agents primarily affect lymphoid cells because these cells contain an enzyme necessary for activation of these compounds. Previous investigations employing these drugs in patients with low grade lymphoid malignancies have shown that they selectively target immune cells by inducing a physiologic form of cell death called apoptosis. Their effects on the clinical course of immunologic diseases, as well as on the function of immune cells have not been characterized.

Twelve patients have been entered into a study for treatment of refractory rheumatoid arthritis and one into a study for the treatment of psoriatic arthritis. To date, no major or unexpected toxicities have been encountered. Fludarabine treatment has resulted in sustained B and T lymphopenia ($B > T$). We are attempting to determine whether the level of lymphopenia should be a major determinant of safe drug doses; no substantive changes in total immunoglobulin or antibody levels have been seen. At the low doses of fludarabine used, significant clinical effects have been observed in eight patients with rheumatoid arthritis who have completed this therapy so far.

ZO1AR41111-01 ARB

Clinical and Therapeutic Studies of the Rheumatic Diseases H.

Schumacher, R

This study proposes to establish a cohort of patients with inflammatory arthritis of less than one year's duration in an attempt to better understand pathogenetic mechanisms involved in early synovitis and to determine prognostic factors associated with the clinical disease course. Synovial biopsies and synovial fluid will be obtained to search for microbial agents and other initiating and modulating factors that may be most readily distinguished early in the disease and to determine the stage of disease at which certain immunologic and hormonal changes become evident. Specimens will be evaluated by polymerase chain reaction and immunoelectron microscopy for the presence of infectious agents such as difficult to culture chlamydia and to assess cytokine profiles present with the synovial membrane. The study will search for genetic, in particular HLA DR loci, and other features that may be associated with specific forms of inflammatory arthropathies that might predict the subsequent clinical disease course or response to different agents used in treatment of rheumatoid arthritis and other types of chronic inflammatory arthropathies. Assessments of the neuroendocrine axis suggest that low cortisol or androgens favor development of rheumatoid arthritis. These studies will contribute to knowledge about the early pathogenic events in early arthritis and have the potential to assist in development of new approaches to patient management.

ZO1AR41112-01 ARB

Treatment & Pathogenesis of Skin Cancer, Disorders of Cornification & Psoriasis
DiGiovanna, JJ

The goal of these studies is to explore the efficacy, toxicity, and mechanisms of action of new treatments, and to better understand the pathogenesis of dermatologic diseases, particularly skin cancer and the disorders of cornification. Patients followed long-term at NIH have established the efficacy and characterized the toxicity of isotretinoin and etretinate for several skin disorders. These patients continue to be monitored to characterize skeletal toxicity, an important chronic side effect. Understanding the scope of chronic retinoid toxicity is important because of the expanding number of synthetic retinoids under clinical study. The peripheral skeletal involvement that frequently occurs after chronic etretinate therapy was first identified in this cohort. We have now discovered that osteoporosis is also a toxicity of etretinate. In patients with Darier's disease, we have identified a novel, common, cystic bone abnormality. Studies directed at skin cancer treatment and prevention are continuing. We demonstrated the effectiveness of oral isotretinoin as a chemopreventive agent in patients with high rates of skin cancer formation. These patients are now maintained on long-term isotretinoin chemoprevention to determine optimum dosing. A recently completed phase I/II study of gamma interferon for basal cell carcinoma demonstrated shrinking of all tumors, but few completely regressed. We have begun a phase I/II study of topical antiflammin, a novel peptide inhibitor of phospholipase A2, which has homology to uteroglobin and lipocortin. Our collaboration with the Lab. of Skin Biology has expanded (ZO1-AR-41089-04 LSB). We were the first to find linkage of the epidermolytic hyperkeratosis gene to the type II keratin gene cluster on chromosome 12q, and have now identified mutations in keratin 1 or 10 in 12 families. Based on our clinical studies we have created a new classification of the disease including 6 distinct clinical phenotypes. Correlation of mutations with clinical phenotypes will lead to a better understanding of the relationship between keratin structure and function. In a pedigree with a non-epidermolytic form of palmar/plantar keratoderma (Unna-Thost disease) we found complete linkage with the type II

keratin region and discovered a mutation in the V1 domain of the keratin 1 gene as the cause of this disease. Our fine mapping study of Darier's disease narrowed the location of the disease gene to a 5 cM interval on chromosome 12q23-q24.1. In lamellar ichthyosis we were first to find linkage to chromosome 14q11 and have now identified mutations in transglutaminase 1 as the cause of the disease. Detailed clinical analysis will allow correlation of mutations with clinical phenotypes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41084-06

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Features of Keratin and Related Intermediate Filaments

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: Peter M. Steinert

Chief, LSB, NIAMS

Others: Eleonora Candi

Special Volunteer, LSB, NIAMS

Constantin Chipev

Visiting Scientist, LSB, NIAMS

James Mack

Special Volunteer, LSB, NIAMS

Lyuben Marekov

Visiting Scientist, LSB, NIAMS

COOPERATING UNITS (if any)

C Cohen, Dept of Biol, Brandeis Univ, Waltham; R Goldman, Dept of Cell, Mol & Struct Biol, Northwestern Univ, Chicago; W Ip, Dept of Cell

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

2.0

2.0

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human ☒ (b) Human ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The structure, function and expression of keratin intermediate filaments of human skin, and the related intermediate filament proteins of other cell types are being investigated. These studies are designed to understand the structural features which determine how the rod domains of the constituent coiled-coil molecules pack together to form the core of the filament, and how the protruding end domains define filament function. Having recently developed detailed models on probable molecular packing, we have constructed a series of synthetic peptides corresponding to important molecular overlap regions for the purpose of X-ray crystallography to solve three dimensional structures. Several of these peptides have been micro-injected into living cells in order to explore their dynamic behavior. In order to explore the functions of end domain sequences, we have constructed another series of peptides to structurally interesting portions of vimentin to explore their protein interactions by way of the yeast two hybrid system. In other studies, we have determined that a portion of the V1 region of the type II keratins 1, 2e and 5 is involved in crosslinking with other proteins to the cornified cell envelope, a process which is likely to provide a mechanism by which the keratin intermediate filament cytoskeleton is anchored to the cell periphery. Analysis of other crosslinks reveals that the keratin chains may also be attached to the desmoplakin component of desmosomes indirectly by way of an intermediate filament associated protein. Epidermal keratinocytes obtained from a patient with epidermolytic hyperkeratosis with a keratin 10 gene mutation have been virally transformed. The cells express both the normal and mutated alleles. This cell line will now be very useful to test gene therapy approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41085-06 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Filaggrin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI: Peter M. Steinert	Chief, LSB, NIAMS
Others: Jeanne Andreoli	IRTA Fellow, LSB, NIAMS
Vincenzo De Laurenzi	Special Volunteer, LSB, NIAMS
Shyh-Ing Jang	IRTA Fellow, LSB, NIAMS
Nedialka Markova	Visiting Scientist, LSB, NIAMS

COOPERATING UNITS (if any)

IG Kim, Dept of Biochemistry, Seoul National University, Seoul, Korea

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

2.5

PROFESSIONAL:

2.5

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Filaggrin, the processed form of profilaggrin, is a major product of terminally differentiating mammalian epidermal cells which, based on in vitro studies, is thought to be involved in the aggregation and specific alignment of keratin intermediate filaments during the final stages of development in vivo. In order to explore this possibility, a vector containing one filaggrin repeat was coupled to an inducible heat shock promoter, and stably transfected keratinocyte cell lines were developed. Upon activation of the promoter, the expressed filaggrin quickly disrupts the keratin cytoskeleton due to aggregation of the filaments in the vicinity of the nucleus by causing their collapse. We have also explored the proximal promoter region of the human profilaggrin gene. This consists of a complex of an Sp1 site modulated by nearby interacting ets, NF-KB and AP-1 like consensus transcription sequences. All of these elements are functional, based on gel shift, foot printing and mutational analyses. The ets element is different from the ets-1 and ets-2 sequences heretofore characterized. Since this same ets motif is present in two other epidermally-expressed genes (transglutaminase 3 and trichohyalin), we have initiated a detailed analysis of the ets-like transcription factors expressed in epidermal keratinocytes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41086-06 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Loricrin, A Major Cell Envelope Protein

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert	Chief, LSB, NIAMS
Others:	Eleonora Candi	Special Volunteer, LSB, NIAMS
	Vincenzo De Laurenzi	Special Volunteer, LSB, NIAMS
	William Idler	Chemist, LSB, NIAMS
	Tonja Kartasova	Guest Researcher, LSB, NIAMS
	Soo-Youl Kim	Visiting Fellow, LSB, NIAMS
	James Mack	Special Volunteer, LSB, NIAMS
	Lyuben Marekov	Visiting Scientist, LSB, NIAMS
	Edit Tarcsa	Visiting Fellow, LSB, NIAMS

COOPERATING UNITS (if any)

Genaro Melino, Giampiero Mei, Department of Experimental Medicine, University Tor Vergata, Rome, Italy

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The cornified cell envelope is a multi-component 15 nm thick layer of highly insoluble protein deposited on the inner surface of the plasma membrane of terminally differentiating epithelial cells. This insolubility is due to crosslinking of the proteins by N^ε-(γ-glutamyl) lysine isodipeptide crosslinks introduced by transglutaminases. In order to determine the structure of the epidermal cornified cell envelope, we have devised digestion protocols with trypsin and proteinase K to release soluble peptides for amino acid microsequencing. Cell envelopes were isolated from fully cornified human foreskin epidermis before and after saponification with methanol/KOH, or cultured keratinocytes. We have now sequenced about 500 peptides from which we can conclude that: loricrin constitutes the bulk of the cell envelope, up to 70%, and is localized primarily on the outer (cytoplasmic surface) two-thirds of the structure; SPR1 and SPR2 proteins are admixed throughout the loricrin phase, and serve as crossbridging proteins amongst the loricrin; the novel protein elafin is also admixed with the loricrin; in the inner portions of the cell envelope structure, corresponding to the earliest stages of its assembly, involucrin and cystatin a are abundant components crosslinked to each other and the loricrin phase; keratin intermediate filament chains are also crosslinked components, but in this case, they are crosslinked indirectly to desmoplakin of the desmosomes, mediated by an intermediate filament associated protein. Full-length human loricrin has been expressed in bacteria and purified to homogeneity. Three epidermally-expressed transglutaminases crosslink it differently in vitro. Based on the patterns of crosslinking, the data suggest that transglutaminase 3, and to a lesser extent transglutaminase 1, are both required to crosslink the loricrin in vivo. We have expressed mouse and human SPR1, SPR2 and SPR3 proteins in bacteria for subsequent biochemical, in vitro crosslinking and structural analyses. In order to define its precise function in the epidermis, we have made constructs to prepare loricrin null mice.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41087-06 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Epidermal Transglutaminases

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert	Chief, LSB,NIAMS
Others:	Vincenzo DeLaurenzi	Special Volunteer, LSB,NIAMS
	William Idler	Chemist, LSB,NIAMS
	Shyh-Ing Jang	IRTA Fellow, LSB,NIAMS
	Jeung-Hoon Lee	Visiting Fellow, LSB,NIAMS
	Lyuben Marekov	Visiting Scientist, LSB, NIAMS
	Nedialka Markova	Visiting Scientist, LSB, NIAMS

COOPERATING UNITS (if any)

Soo-Il Chung, Korean Green Cross Corporation, Korea; Soo-Youl Kim, Pacific Research Corporation, Korea

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, Bethesda, MD 20892

TOTAL STAFF YEARS:

PROFESSIONAL:

OTHER:

2.5

2.5

0.5

CHECK APPROPRIATE BOX(IES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Transglutaminases form an isodipeptide crosslink between an acceptor amide group of a protein bound glutamine residue and a donor ϵ -NH₂ of a protein bound lysine residue, thereby forming a highly insoluble macromolecular complex. In the epidermis, three enzymes are thought to be involved in crosslinking of certain defined structural proteins to form an insoluble cornified cell envelope, and are transglutaminases 1, 2 and 3. We are studying in detail the roles of the human transglutaminase 1 and 3 enzymes. By use of new specific antibodies, we have found that the transglutaminase 1 system in keratinocytes or foreskin epidermis is very complex, since it exists in multiple soluble as well as membrane-bound forms. The most active of these, a 67 and 33kDa complex held together by secondary forces, is generated during terminal differentiation in these cells by proteolytic cleavage of specific sites that are conserved in the family of transglutaminases. This is similar to the known proteolytic activation of the transglutaminase 3 system. The proximal promoter region of the transglutaminase 3 gene is located within the first 125 bp above the transcription initiation start site, and consists of an Sp1 motif modulated by two adjacent ets-like motifs. These are sufficient to connote epithelial specific expression to this gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41089-04 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Genetic Studies of Hereditary Skin Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
P.I.: S Bale, Chief, GSS, LSB

Others:

Y Whyte, Pre-doctoral IRTA, GSS,LSB,NIAMS

L Russell, Sr. Staff Fellow, GSS, NIAMS

J DiGiovanna, Sr. Staff Fellow, CDIU,IRP,NIAMS

J Compton, Staff Scientist, GSS,LSB,NIAMS

P Steinert, Lab Chief, LSB,NIAMS

G Rogers, Visiting Fellow

C Chipev, Visiting Scientist, LSB, NIAMS

V Kimonis, Visiting Fellow, GSS,LSB,NIAMS

J Lin, IRTA Fellow, GSS,LSB,NIAMS

COOPERATING UNITS (if any)

N Hashem, Professor, Ain-Shams, Medical Genetic Center, Cairo, Egypt; C Mazzanti, Instituto Dermapatico dell'Immacolata, Rome, Italy; W Rizzo, Professor, Medical College of Virginia, Richmond

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

4.1

PROFESSIONAL:

2.5

OTHER:

1.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We are studying the genetic basis of a variety of skin disorders, including epidermolytic hyperkeratosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, Darier's disease, Hailey-Hailey disease, erythrokeratoderma variabilis, white sponge nevus, Sjogren-Larsson syndrome and Gorlin syndrome. Patients and their families are recruited for study. They travel to the NIH clinics (or rarely we travel to them) to enable our research group to perform detailed family and medical histories, skin examinations, skin biopsies, phlebotomy, and clinical photography. DNA is extracted from patient's and family member's buccal cells. Skin samples are used to confirm diagnoses and to investigate ultrastructural abnormalities specific to each disease. Clinical heterogeneity (different clinical appearances of the "same" disease) is investigated using the information collected. DNA-based polymorphisms are used for linkage studies to determine the chromosomal location of the skin disease locus. Identification of the disease-causing gene is made by searching for mutations in candidate genes in the mapped regions. Genotype-phenotype correlations are drawn based on the clinical presentation and the specific gene mutation.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41090-04 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Consultation for Genetic Analyses and Development of Statistical Methods

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) laboratory,
P.I.: SJ Bale, Chief, GSS,LSB,NIAMS

Others: J Lin, IRTA Fellow, GSS,LSB,NIAMS

COOPERATING UNITS (if any)

CI Amos, MD Anderson Cancer Center, Houston, Texas

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Genetic Studies Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

1 1

PROFESSIONAL:

1 1

OTHER:

0 0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

NOTE: This project has been combined with Z01-AR-41091 and is now called "Consultation for Genetic Analyses and Development of Statistical Methods"

We continue to provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases, and we develop new methods of analysis where needed to fill the needs of these investigators. We have again worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 41093-04 LSB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Expression, Structure and Function of Trichohyalin

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

PI:	Peter M. Steinert	Chief, LSB,NIAMS
Others:	Jeanne Andreoli	IRTA Fellow, LSB, NIAMS
	William Idler	Chemist, LSB, NIAMS
	Shyh-Ing Jang	IRTA Fellow, LSB, NIAMS
	Lyuben Marekov	Visiting Scientist, LSB,NIAMS
	Nedialka Markova	Visiting Scientist, LSB,NIAMS
	Edit Tarcsa	Visiting Fellow, LSB,NIAMS

COOPERATING UNITS (if any)

Soo-II Chung, Korean Green Cross Corporation, Korea; Genaro Melino, Giampiero Mei, Department of Experimental Medicine, University Tor Vegata, Rome, Italy; Seung-Chul

LAB/BRANCH

Laboratory of Skin Biology

SECTION

Molecular Biology of Keratinization Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

3.0

PROFESSIONAL:

3.0

OTHER:

0.5

CHECK APPROPRIATE BOXES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Trichohyalin is a major differentiation product of the inner root sheath cells of hair follicle, the medulla of the hair fiber, and is expressed in minor amounts in the epidermis. It is thought to function as a keratin intermediate filament associated protein in these tissues. In addition, trichohyalin is a substrate for transglutaminases (TGases), and for the enzyme peptidylarginine deiminase (PAD) which can convert arginines into citrullines. We have expressed domain 8 of human trichohyalin in bacteria, purified it to homogeneity, and used it as a substrate for TGases and PAD. PAD can convert up to 60% of the arginines of trichohyalin into citrullines. In another PAD substrate, filaggrin, the conversion efficiency is near 100%. By use of a series of model proteins and peptide substrates, the reaction by PAD is clearly related to structure. By circular dichroism and fluorescence spectroscopy, the trichohyalin is a highly ordered ~90% α -helical protein in solution. Following reaction with PAD, all organized secondary structure is lost, presumably because the substituted urea derivative (citrulline) interferes with protein structure. The trichohyalin is used as a substrate by the three TGases expressed in the skin, but by calculation of kinetic parameters, TGase 3 uses it most efficiently. Multiple glutamines and lysines are utilized, indicating that trichohyalin is a complete TGase substrate. The K_m for the TGase 3 enzyme increases by a factor of 10 after PAD modification. These data suggest that the highly insoluble trichohyalin is first modified to a soluble form in cells by PAD before it is preferentially crosslinked by TGase 3. An antibody elicited against the expressed trichohyalin is being used to explore tissue expression. An analysis of the regulatory sequences which control expression of the trichohyalin gene have identified several sequences within the first 150 bp above the transcription start site which define the proximal promoter and epithelial-specific expression. However, 1.5 kb of upstream sequences coupled to a β -galactosidase reporter gene are required to connote expression in transgenic mice in the hair follicle cells, hard palate, tongue epithelium and nail beds.

Z01AR41084-06 LSB

Structural Features of Keratin and Related Intermediate Filaments
Steinert, PM

The structure, function and expression of keratin intermediate filaments of human skin, and the related intermediate filament proteins of other cell types are being investigated. These studies are designed to understand the structural features which determine how the rod domains of the constituent coiled-coil molecules pack together to form the core of the filament, and how the protruding end domains define filament function. Having recently developed detailed models on probable molecular packing, we have constructed a series of synthetic peptides corresponding to important molecular overlap regions for the purpose of X-ray crystallography to solve three dimensional structures. Several of these peptides have been micro-injected into living cells in order to explore their dynamic behavior. In order to explore the functions of end domain sequences, we have constructed another series of peptides to structurally interesting portions of vimentin to explore their protein interactions by way of the yeast two hybrid system. In other studies, we have determined that a portion of the V1 region of the type II keratins 1, 2e and 5 is involved in crosslinking with other proteins to the cornified cell envelope, a process which is likely to provide a mechanism by which the keratin intermediate filament cytoskeleton is anchored to the cell periphery. Analysis of other crosslinks reveals that the keratin chains may also be attached to the desmoplakin component of desmosomes indirectly by way of an intermediate filament associated protein. Epidermal keratinocytes obtained from a patient with epidermolytic hyperkeratosis with a keratin 10 gene mutation have been virally transformed. The cells express both the normal and mutated alleles. This cell line will now be very useful to test gene therapy approaches.

Z01AR41085-06 LSB

Expression, Structure and Function of Filaggrin
Steinert, PM

Filaggrin, the processed form of profilaggrin, is a major product of terminally differentiating mammalian epidermal cells which, based on in vitro studies, is thought to be involved in the aggregation and specific alignment of keratin intermediate filaments during the final stages of development in vivo. In order to explore this possibility, a vector containing one filaggrin repeat was coupled to an inducible heat shock promoter, and stably transfected keratinocyte cell lines were developed. Upon activation of the promoter, the expressed filaggrin quickly disrupts the keratin cytoskeleton due to aggregation of the filaments in the vicinity of the nucleus by causing their collapse. We have also explored the proximal promoter region of the human profilaggrin gene. This consists of a complex of an SpI site modulated by nearby interacting ets, NF-KB and AP-1 like consensus transcription sequences. All of these elements are functional, based on gel shift, foot printing and mutational analyses. The ets element is different from the ets-1 and ets-2 sequences heretofore characterized. Since this same ets motif is present in two other epidermally-expressed genes (transglutaminase 3 and trichohyalin), we have initiated a detailed analysis of the ets-like transcription factors expressed in epidermal keratinocytes.

Z01AR41086-06 LSB

Expression, Structure and Function of Loricrin, A Major Cell Envelope Protein
Steinert, PM

The cornified cell envelope is a multi-component 15 nm thick layer of highly insoluble protein deposited on the inner surface of the plasma membrane of terminally differentiating epithelial cells. This insolubility is due to crosslinking of the proteins by N^ε-(γ-glutamyl) lysine isodipeptide crosslinks introduced by transglutaminases. In order to determine the structure of the epidermal cornified cell envelope, we have devised digestion protocols with trypsin and proteinase K to release soluble peptides for amino acid microsequencing. Cell envelopes were isolated from fully cornified human foreskin epidermis before and after saponification with methanol/KOH, or cultured keratinocytes. We have now sequenced about 500 peptides from which we can conclude that: loricrin constitutes the bulk of the cell envelope, up to 70%, and is localized primarily on the outer (cytoplasmic surface) two-thirds of the structure; SPR1 and SPR2 proteins are admixed throughout the loricrin phase, and serve as crossbridging proteins amongst the loricrin; the novel protein elafin is also admixed with the loricrin; in the inner portions of the cell envelope structure, corresponding to the earliest stages of its assembly, involucrin and cystatin a are abundant components crosslinked to each other and the loricrin phase; keratin intermediate filament chains are also crosslinked components, but in this case, they are crosslinked indirectly to desmoplakin of the desmosomes, mediated by an intermediate filament associated protein. Full-length human loricrin has been expressed in bacteria and purified to homogeneity. Three epidermally-expressed transglutaminases crosslink it differently in vitro. Based on the patterns of crosslinking, the data suggest that transglutaminase 3, and to a lesser extent transglutaminase 1, are both required to crosslink the loricrin in vivo. We have expressed mouse and human SPR1, SPR2 and SPR3 proteins in bacteria for subsequent biochemical, in vitro crosslinking and structural analyses. In order to define its precise function in the epidermis, we have made constructs to prepare loricrin null mice.

Z01AR41087-06 LSB

Epidermal Transglutaminases
Steinert, PM

Transglutaminases form an isodipeptide crosslink between an acceptor amide group of a protein bound glutamine residue and a donor ε-NH₂ of a protein bound lysine residue, thereby forming a highly insoluble macromolecular complex. In the epidermis, three enzymes are thought to be involved in crosslinking of certain defined structural proteins to form an insoluble cornified cell envelope, and are transglutaminases 1, 2 and 3. We are studying in detail the roles of the human transglutaminase 1 and 3 enzymes. By use of new specific antibodies, we have found that the transglutaminase 1 system in keratinocytes or foreskin epidermis is very complex, since it exists in multiple soluble as well as membrane-bound forms. The most active of these, a 67 and 33kDa complex held together by secondary forces, is generated during terminal differentiation in these cells by proteolytic cleavage of specific sites that are conserved in the family of transglutaminases. This is similar to the known proteolytic activation of the transglutaminase 3 system. The proximal promoter region of the transglutaminase 3 gene is located within the first 125 bp above the transcription initiation start site, and consists of an Sp1 motif modulated by two

adjacent ets-like motifs. These are sufficient to connote epithelial specific expression to this gene.

Z01AR41089-04 LSB

Genetic Studies of Hereditary Skin Disorders

Bale, SJ

We are studying the genetic basis of a variety of skin disorders, including epidermolytic hyperkeratosis, lamellar ichthyosis and congenital ichthyosiform erythroderma, Darier's disease, Hailey-Hailey disease, erythrokeratoderma variabilis, white sponge nevus, Sjogren-Larsson syndrome and Gorlin syndrome. Patients and their families are recruited for study. They travel to the NIH clinics (or rarely we travel to them) to enable our research group to perform detailed family and medical histories, skin examinations, skin biopsies, phlebotomy, and clinical photography. DNA is extracted from patient's and family member's buccal cells. Skin samples are used to confirm diagnoses and to investigate ultrastructural abnormalities specific to each disease. Clinical heterogeneity (different clinical appearances of the "same" disease) is investigated using the information collected. DNA-based polymorphisms are used for linkage studies to determine the chromosomal location of the skin disease locus. Identification of the disease-causing gene is made by searching for mutations in candidate genes in the mapped regions. Genotype-phenotype correlations are drawn based on the clinical presentation and the specific gene mutation.

Z01AR41090-04 LSB

Consultation for Genetic Analyses and Development of Statistical Methods

Bale, SJ

We continue to provide consultation and support (analytic, computer software, computation) for various investigators, both intramural and extramural, who are interested in assessing the genetic component of diseases, and we develop new methods of analysis where needed to fill the needs of these investigators. We have again worked this fiscal year with several scientists, both intramural (NIAMS and other NIH institutes) and extramural (both NIH and outside institutions) to assist in the design and execution of studies to 1) assess familial aggregation of disease, 2) investigate linkage relationships between disease and genetic markers, 3) assess the relative risks of various environmental components to the development of disease, and 4) provide software support for genetic analysis programs.

Z01AR41093-04 LSB

Expression, Structure and Function of Trichohyalin

Steinert, PM

Trichohyalin is a major differentiation product of the inner root sheath cells of hair follicle, the medulla of the hair fiber, and is expressed in minor amounts in the epidermis. It is thought to function as a keratin intermediate filament associated protein in these tissues. In addition, trichohyalin is a substrate for transglutaminases (TGases), and for the enzyme peptidylarginine deiminase (PAD) which can convert arginines into citrullines. We have expressed domain 8 of

human trichohyalin in bacteria, purified it to homogeneity, and used it as a substrate for TGases and PAD. PAD can convert up to 60% of the arginines of trichohyalin into citrullines. In another PAD substrate, filaggrin, the conversion efficiency is near 100%. By use of a series of model proteins and peptide substrates, the reaction by PAD is clearly related to structure. By circular dichroism and fluorescence spectroscopy, the trichohyalin is a highly ordered $\sim 90\%$ α -helical protein in solution. Following reaction with PAD, all organized secondary structure is lost, presumably because the substituted urea derivative (citrulline) interferes with protein structure. The trichohyalin is used as a substrate by the three TGases expressed in the skin, but by calculation of kinetic parameters, TGase 3 uses it most efficiently. Multiple glutamines and lysines are utilized, indicating that trichohyalin is a complete TGase substrate. The k_{cat} for the TGase 3 enzyme increases by a factor of 10 after PAD modification. These data suggest that the highly insoluble trichohyalin is first modified to a soluble form in cells by PAD before it is preferentially crosslinked by TGase 3. An antibody elicited against the expressed trichohyalin is being used to explore tissue expression. An analysis of the regulatory sequences which control expression of the trichohyalin gene have identified several sequences within the first 150 bp above the transcription start site which define the proximal promoter and epithelial-specific expression. However, 1.5 kb of upstream sequences coupled to a β -galactosidase reporter gene are required to connote expression in transgenic mice in the hair follicle cells, hard palate, tongue epithelium and nail beds.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27001-21 LPB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Muscle Contractility and Regulation

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**
Mark Schoenberg, M.D., Medical Officer, LPB, NIAMS

COOPERATING UNITS (if any)

Dr. Vincent Barnett
University of Minnesota Medical School, Minneapolis, MN 55455

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Treating muscle crossbridges in the presence of ATP with the alkylating agent N-phenylmaleimide (NPM) links phenylmaleimide groups to Cys-707 and Cys-697 on myosin heavy chain and totally inhibits the crossbridges' ability to make force. Force is inhibited by NPM because the crossbridges are prevented from undergoing the weakly-binding to strongly-binding conformational change. Treating crossbridges with fluorodinitrobenzine (FDNB) in the presence of pyrophosphate links a dinitrophenyl to Cys-707 and inhibits force by 90%, but it does so by a different mechanism than NPM since the dinitrophenylated crossbridge is still capable of undergoing the weak-to-strong transition. In terms of attachment and detachment rate constants, the weakly- and strongly-binding crossbridge states of the dinitrophenylated crossbridges are very similar to those of normal crossbridges.

Treating the FDNB-treated crossbridges with NPM, and then removing the dinitrophenyl group using dithiothreitol creates a third type of crossbridge, one with a single phenylmaleimide linked to Cys-697. These crossbridges behave similarly to those having phenylmaleimide at both reactive sulfhydryls, suggesting that having just a single phenylmaleimide at Cys-697 is sufficient to prevent the weak-to-strong crossbridge conformational change.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27003-36 LPB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biophysical Studies of Metabolic Activity and Control

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory, Ellis S. Kempner, Ph.D., Physicist and Chief, Section on Macromolecular Biophysics, LPB, NIAMS**

COOPERATING UNITS (if any)

Drs. E. Dennis (UCSD); C. Hirschberg (Univ. Mass)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Section on Macromolecular Biophysics

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda Maryland 20892

TOTAL STAFF YEARS:

2.0

PROFESSIONAL:

1.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Biologically active materials exposed to high energy electrons are damaged. If the irradiation is carried out at very low temperatures, the rates of loss of activity or structure with radiation dose are directly proportional to the mass of the molecules which are required in the assay. the presence of other molecules has no effect on the measurement of mass; purified samples are not required.

Target theory of the inactivation of biological activity by ionizing radiation was extended to several complex biochemical systems. Target size analysis has been applied to several major problems including the molecular forms of extracellular phospholipases A₂ and various Golgi enzymes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27004-26 LPB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Dynamic Properties of Cell Membranes and Related Systems

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**
Norman L. Gershfeld, Ph.D. Research Chemist LPB, NIAMS

COOPERATING UNITS (if any)

Dr. L. Ginsberg, Dept. of Neurological Sciences, Royal Free Hospital School of Medicine, University of London; Dr. C.P. Mudd and T.L. Talbot, ACES, BEIP, NCRR; Dr. S.I. Rapoport, LN, NIA

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Macromolecular Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, MD 20892

TOTAL STAFF YEARS:

2.25

PROFESSIONAL:

1.0

OTHER:

1.25

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanisms of membrane assembly, and the conditions which influence membrane stability in normal and diseased cells are the principal focus of this program. We have previously reported that cell membranes assemble and are optimally stable at a critical temperature T_p , the physiological temperature of the cell; the lipid composition of the membrane bilayer is the principal determinant of the critical temperature. The conditions for membrane assembly and stability may be simulated in aqueous dispersions of the total lipid extracts of membranes. Thus, for normal, disease-free cells the critical temperatures measured in these aqueous dispersions have been shown to equal T_p . A rapid technique for this measurement has been developed and applied to the total membrane lipid extracts from human erythrocytes (37.2°C), rat spinal cord and cerebrum (39.0°C), rabbit erythrocytes (39.0°C) and cerebellum (39.4°C), in general agreement with reported values for T_p . Destabilization of cell membranes, and membrane breakdown may occur when the lipid composition is altered by metabolism such that the critical temperature of the membrane is less than T_p . This concept has been utilized to identify a lipid defect that is site-specific in post-mortem brain samples from patients with Alzheimer's disease. The defect is manifested as a deficit in ethanolamine plasmalogen in mid-temporal cortex with marked neurodegeneration, in contrast to normal values in regions that are disease-free.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27012-10 LPB

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural and Mechanical Properties of Muscle Fibers

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**

P.I.: Leepo C. Yu, Ph.D. Research Physicist LPB, NIAMS

Others: Sengen Xu, Ph.D. Visiting Scientist LPB, NIAMS

Sergey Malinchik, Ph.D. Visiting Associate LPB, NIAMS

Suzanne Frisbie, Ph.D. IRTA LPB, NIAMS

Terry Kraft, Ph.D. Visiting Fellow LPB, NIAMS

COOPERATING UNITS (if any)

Medical School of Hannover, FRG (Dr. B. Brenner); East Carolina University Medical School, North Carolina (Dr. J. Chalovich); Duke University (M. Reedy); NIDDK/NIH, (Dr. Helmut Strey)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

5.0

PROFESSIONAL:

5.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The long term goal of this program is to identify and characterize the *in vivo* structures of the contractile proteins during various phases of contraction. Previously we have identified that myosin heads (cross-bridges) must first attach to actin in a weakly bound, non-force-generating configuration. We have proposed that the generation of isometric force results from a structural change in the cross-bridges making a transition from the weakly bound configuration to a strongly bound, force generating configuration. The focus of our research has been to investigate both experimentally and by modelling, the molecular structures of the two types of cross-bridge configurations.

We have proposed that the cross-bridges weakly bound to actin assume a flexible, (non-stereo-specific) configuration; while those strongly bound assume a rigid (stereo-specific) configuration. In FY 95 we have developed computational models to derive expected diffraction patterns from three different configurations of the cross-bridges: when there is no interaction between actin and myosin; when the binding is non-stereo-specific; and when the binding is stereo-specific. Calculations suggest that the non-stereo-specific binding of myosin heads to actin is characterized by the appearance of a mixture of myosin and actin based layer lines, instead of myosin layer lines being dominating as in the case of no interaction. The stereo-specific binding is characterized by the disappearance of myosin-based layer lines while the actin based layer lines become dominant. In FY95 we have obtained high resolution diffraction patterns, by using synchrotron radiation sources, from relaxed and rigor fibers. Experimental diffraction patterns can now be compared with the calculated ones to derive proper interpretation of the experimental data.

The structure of weakly bound cross-bridges in the presence of calcium was investigated, since several models on contraction mechanism suggest that activation by calcium induces an intermediate structural state in the pathway to force generation. Two dimensional diffraction patterns were obtained from single fibers using ATPgS in the presence and in the absence of calcium. Preliminary results showed that apart from the changes related to the activation process, there is no large scale structural changes upon addition of calcium. This finding had direct implications for our understanding of the pathway to force generation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27014-04 LPB

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Molecular Mechanisms of Myofibril Assembly and Function

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation) **laboratory,**

Robert Horowitz, Ph.D. Research Biologist

Gang Luo, Ph.D. IRTA Fellow

Jin Qiao Zhang, Ph.D. Visiting Fellow

Amy Herrera, M.S. Biologist

Stanley V. Smith, Ph.D. IRTA Fellow

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Physical Biology

SECTION

Muscle Biophysics Section

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda MD 20892

TOTAL STAFF YEARS:

3.6

PROFESSIONAL:

3.1

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The processes involved in the assembly of complex, multimolecular biological structures are poorly understood. This project seeks to use the striated muscle myofibril as a model system in which to explore these mechanisms.

Extremely large myofibrillar proteins are hypothesized to play a key role in myofibril assembly. As a first step to studying the function of extremely large myofibrillar proteins using molecular genetic approaches, we are cloning, sequencing, and expressing portions of the mouse nebulin message. To date, we have characterized three clones encoding the central third of the 800 kDa nebulin protein. The three clones can be assembled into two distinct contiguous sequences that encode different isoforms of the protein. The translated sequence consists of a series of 245 residue repeats, in agreement with the published sequence of human nebulin. The translated mouse nebulin sequence is greater than 90% identical to the human sequence, with the exception of a 200 base pair region where the level of identity drops to 58%. We have constructed prokaryotic expression vectors carrying two of the isolated clones, and have succeeded in expressing 115,000 and 75,000 dalton fragments to high levels in *E. coli*. We are currently purifying the expressed nebulin fragments for use in in vitro experiments.

In the course of this project, we had discovered a novel, muscle specific message with homology to nebulin. We have now cloned and sequenced over 90% of this protein. The sequence data predict a - 115,000 dalton protein, with two nebulin-like 245 residue repeats near the C-terminus. We have produced an antibody against a synthetic peptide derived from the deduced amino acid sequence of this cDNA. In immunoblots, this antibody detects expressed fragments of the nebulin related protein in *E. coli*, and a single protein of the predicted size in mouse skeletal muscle. A slightly smaller band is detected in mouse cardiac muscle, indicating the presence of tissue specific isoforms of the nebulin related protein. These results also confirm our previous conclusion, based on northern blot experiments, that the nebulin related protein is expressed in both skeletal and cardiac muscle. We are currently purifying this novel protein for use in in vitro experiments, as well as using the specific antibody to localize it in the muscle cell.

Z01AR27001-21 LPB

Muscle Contractility and Regulation

Schoenberg, M

Treating muscle crossbridges in the presence of ATP with the alkylating agent N-phenylmaleimide (NPM) links phenylmaleimide groups to Cys-707 and Cys-697 on myosin heavy chain and totally inhibits the crossbridges' ability to make force. Force is inhibited by NPM because the crossbridges are prevented from undergoing the weakly-binding to strongly-binding conformational change. Treating crossbridges with fluorodinitrobenzine (FDNB) in the presence of pyrophosphate links a dinitrophenyl to Cys-707 and inhibits force by 90%, but it does so by a different mechanism than NPM since the dinitrophenylated crossbridge is still capable of undergoing the weak-to-strong transition. In terms of attachment and detachment rate constants, the weakly- and strongly-binding crossbridge states of the dinitrophenylated crossbridges are very similar to those of normal crossbridges.

Treating the FDNB-treated crossbridges with NPM, and then removing the dinitrophenyl group using dithiothreitol creates a third type of crossbridge, one with a single phenylmaleimide linked to Cys-697. These crossbridges behave similarly to those having phenylmaleimide at both reactive sulfhydryls, suggesting that having just a single phenylmaleimide at Cys-697 is sufficient to prevent the weak-to-strong crossbridge conformational change.

Z01AR27003-36 LPB

Biophysical Studies of Metabolic Activity and Control

Kempner, ES

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Target theory of the inactivation of biological activity by ionizing radiation was extended to several complex biochemical systems. Target size analysis has been applied to several major problems including the molecular forms of extracellular phospholipases A₂ and various Golgi enzymes.

Z01AR27004-26 LPB

The Dynamic Properties of Cell Membranes and Related Systems

Gershfeld, NL

The mechanisms of membrane assembly, and the conditions which influence membrane stability in normal and diseased cells are the principal focus of this program. We have previously reported that cell membranes assemble and are optimally stable at a critical temperature T_p , the physiological temperature of the cell; the lipid composition of the membrane bilayer is the principal determinant of the critical temperature. The conditions for membrane assembly and

stability may be simulated in aqueous dispersions of the total lipid extracts of membranes. Thus, for normal, disease-free cells the critical temperatures measured in these aqueous dispersions have been shown to equal T_p . A rapid technique for this measurement has been developed and applied to the total membrane lipid extracts from human erythrocytes (37.2°C), rat spinal cord and cerebrum (39.0°C), rabbit erythrocytes (39.0°C) and cerebellum (39.4°C), in general agreement with reported values for T_p . Destabilization of cell membranes, and membrane breakdown may occur when the lipid composition is altered by metabolism such that the critical temperature of the membrane is less than T_p . This concept has been utilized to identify a lipid defect that is site-specific in post-mortem brain samples from patients with Alzheimer's disease. The defect is manifested as a deficit in ethanolamine plasmalogen in mid-temporal cortex with marked neurodegeneration, in contrast to normal values in regions that are disease-free.

Z01AR27012-10 LPB

Structural and Mechanical Properties of Muscle Fibers

Yu, LC

The long term goal of this program is to identify and characterize the in vivo structures of the contractile proteins during various phases of contraction. Previously we have identified that myosin heads (cross-bridges) must first attach to actin in a weakly bound, non-force-generating configuration. We have proposed that the generation of isometric force results from a structural change in the cross-bridges making a transition from the weakly bound configuration to a strongly bound, force generating configuration. The focus of our research has been to investigate both experimentally and by modelling, the molecular structures of the two types of cross-bridge configurations.

We have proposed that the cross-bridges weakly bound to actin assume a flexible, (non-stereo-specific) configuration; while those strongly bound assume a rigid (stereo-specific) configuration. In FY 95 we have developed computational models to derive expected diffraction patterns from three different configurations of the cross-bridges: when there is no interaction between actin and myosin; when the binding is non-stereo-specific; and when the binding is stereo-specific. Calculations suggest that the non-stereo-specific binding of myosin heads to actin is characterized by the appearance of a mixture of myosin and actin based layer lines, instead of myosin layer lines being dominating as in the case of no interaction. The stereo-specific binding is characterized by the disappearance of myosin-based layer lines while the actin based layer lines become dominant. In FY95 we have obtained high resolution diffraction patterns, by using synchrotron radiation sources, from relaxed and rigor fibers. Experimental diffraction patterns can now be compared with the calculated ones to derive proper interpretation of the experimental data.

The structure of weakly bound cross-bridges in the presence of calcium was investigated, since several models on contraction mechanism suggest that activation by calcium induces an intermediate structural state in the pathway to force generation. Two dimensional diffraction patterns were obtained from single fibers using ATPgS in the presence and in the absence of calcium. Preliminary results showed that apart from the changes related to the activation

process, there is no large scale structural changes upon addition of calcium. This finding had direct implications for our understanding of the pathway to force generation.

Z01AR27014-04 LPB

Molecular Mechanisms of Myofibril Assembly and Function

Horowitz, R

The processes involved in the assembly of complex, multimolecular biological structures are poorly understood. This project seeks to use the striated muscle myofibril as a model system in which to explore these mechanisms.

Extremely large myofibrillar proteins are hypothesized to play a key role in myofibril assembly. As a first step to studying the function of extremely large myofibrillar proteins using molecular genetic approaches, we are cloning, sequencing, and expressing portions of the mouse nebulin message. To date, we have characterized three clones encoding the central third of the 800 kDa nebulin protein. The three clones can be assembled into two distinct contiguous sequences that encode different isoforms of the protein. The translated sequence consists of a series of 245 residue repeats, in agreement with the published sequence of human nebulin. The translated mouse nebulin sequence is greater than 90% identical to the human sequence, with the exception of a 200 base pair region where the level of identity drops to 58%. We have constructed prokaryotic expression vectors carrying two of the isolated clones, and have succeeded in expressing 115,000 and 75,000 dalton fragments to high levels in *E. coli*. We are currently purifying the expressed nebulin fragments for use in in vitro experiments.

In the course of this project, we had discovered a novel, muscle specific message with homology to nebulin. We have now cloned and sequenced over 90% of this protein. The sequence data predict a ~115,000 dalton protein, with two nebulin-like 245 residue repeats near the C-terminus. We have produced an antibody against a synthetic peptide derived from the deduced amino acid sequence of this cDNA. In immunoblots, this antibody detects expressed fragments of the nebulin related protein in *E. coli*, and a single protein of the predicted size in mouse skeletal muscle. A slightly smaller band is detected in mouse cardiac muscle, indicating the presence of tissue specific isoforms of the nebulin related protein. These results also confirm our previous conclusion, based on northern blot experiments, that the nebulin related protein is expressed in both skeletal and cardiac muscle. We are currently purifying this novel protein for use in in vitro experiments, as well as using the specific antibody to localize it in the muscle cell.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27013-02 LSBR

PERIOD COVERED

October 1, 1994 through September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

High-Resolution Structure and Function of Biological Macromolecules

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: C. Craig Hyde, Expert, Laboratory of Structural Biology Research, NIAMS

Others:

Patricia Bossart-Whitaker, IRTA Fellow
Steven L. Edwards, Sr. Staff Fellow
Joseph P. Mack, Sr. Staff Fellow
Timothy C. Mueser, IRTA Fellow

COOPERATING UNITS (if any)

LBP/NIDDK(Nancy Nossal), DCBDC/NCI (Michael Maurizi), ARB/NIAMS (Paul Plotz),
PEL/OD (Paul Wingfield, Stephen Stahl), Salk Institute (Frederick Bushman)

LAB/BRANCH

Laboratory of Structural Biology Research

SECTION

Hyde Working Group

INSTITUTE AND LOCATION

NIAMS, NIH, BETHEDSA, MD 20892

TOTAL STAFF YEARS:

4.7

PROFESSIONAL:

4.7

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

This Working Group studies the high-resolution structure and function of proteins and other macromolecules using x-ray diffraction methods. Although the groups' research interests are broadly based in all aspects of protein and nucleic acid structure, a common theme among the research subjects are macromolecular assemblies and complex interacting systems. Other aspects of the work are directed in basic science research on the structure of retroviral proteins and host cellular factors involved in HIV expression. Work on the bacterial CLP P complex, part of a larger ATP-dependent protease complex, has resulted in a better crystal form containing only one 14-mer in the crystallographic asymmetric unit which diffracts to higher resolution. Crystallization studies of human histidyl tRNA synthetase, an autoantigen implicated in polymyositis, has resulted in two new crystal forms. Neither diffracts to high resolution and both contain many copies of the 50 kD protein subunit per crystallographic asymmetric unit. More progress has been made in our studies of the DNA "polymerosome" from T4 phage. We have determined and refined the structures of three forms of the 5'->3' exonuclease ("RNase H"). The protein has structural features in common with a variety of nuclease and recombinases, but possesses a unique, new folding topology. Crystallization efforts are continuing on other members of this system including the polymerase and helicase. Two crystal forms and native and heavy-atom derivative data sets have been obtained for the small GP 59 helicase assembly factor. Our retroviral work has focused in two areas. Several forms of retroviral integrases have been expressed, purified and tested for crystallization. And several members of the host cell NFkB family, including p65-rel, I kappa-B and BCL-3 have likewise been expressed, purified and tested for feasibility in crystallization.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 AR 27002-17 LSBR

PERIOD COVERED

October 1, 1994 to September 30, 1995

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Biology of Macromolecular Assembly

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title,

PI: ALASDAIR C. STEVEN, CHIEF, LSBR, NIAMS

Fabienne Beuron, Daniel Thomas - (Special Volunteer)

Eva Kocsis, Naiqian Cheng, James Conway - (Visiting Associate)

Frank Booy - (Visiting Scientist) - Benes Trus - (Guest Worker)

Bernard Kim, Kevin Ross - (Pre-IRTA Fellow)

Jose Caston, Norman Watts, Michal Jarnik - (Visiting Fellow)

Mario Cerritelli - (IRTA Fellow) - Adam Zlotnick - (NRC Fellow)

COOPERATING UNITS (if any)

J. Wall, J. Hainfeld (Brookhaven); J. Brown, W. Newcomb (Univ. Virginia);

LAB/BRANCH

Laboratory of Structural Biology Research

SECTION

Section on Structural Biology

INSTITUTE AND LOCATION

NIAMS, NIH, Bethesda, Maryland 20892

TOTAL STAFF YEARS:

16

PROFESSIONAL:

14

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human ☐ (b) Human ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This Laboratory seeks to elucidate the mechanisms that control the assembly of macromolecular complexes, with particular emphasis on their functional rationale. Over the past year, a major effort has been directed towards enhancing the resolution of three-dimensional density maps of icosahedral virus capsids calculated from cryo-electron micrographs. Thus, we have characterized the events whereby limited proteolysis of the major capsid protein of bacteriophage HK97 induces the stabilizing expansion of the capsid. The excised domain resides on the inner surface of the hexons and pentons, and its removal permits the prohead to maturation transformation to proceed. This transition facilitates the formation of covalent cross-links between neighboring subunits. (ii) In bovine papillomavirus, the analysis has been extended to a resolution of better than 1nm, revealing a wealth of detail concerning the structures of the pentameric capsomers, and disclosing the possible location of the minor capsid protein, L2. (iii) Capsids of herpes simplex virus assembled from proteins synthesized in insect cells from baculovirus expression vectors have been found to be structurally authentic. The 12kDa VP26 protein has been shown to bind to the major capsid protein VP5 only in its hexon state, not in its penton state. From image analysis of negatively stained specimens of the ClpAP energy-dependent protease, the oligomeric natures of the subcomplexes have been defined, as has their mode of interaction. ClpP, the protease, consists of two 7-fold rings, and ClpA, the ATP-hydrolyzing component, forms hexameric rings. In active complexes, they stack axially, resulting in a symmetry mismatch that may have connotations of mutual rotational movement. Progress has also been made on the assembly of cornified cell envelopes of terminally differentiated keratinocytes, and on the domain organization of bacteriophage tail-fiber proteins.

Z01AR27002-17 LSBR

Structural Biology of Macromolecular Assembly

Steven, AC

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Z01AR27013-02 LSBR

High-Resolution Structure and Function of Biological Macromolecules

Hyde, CC

This Working Group studies the high-resolution structure and function of proteins and other macromolecules using x-ray diffraction methods. Although the groups' research interests are broadly based in all aspects of protein and nucleic acid structure, a common theme among the research subjects are macromolecular assemblies and complex interacting systems. Other aspects of the work are directed in basic science research on the structure of retroviral proteins and host cellular factors involved in HIV expression. Work on the bacterial CLP P complex, part of a larger ATP-dependent protease complex, has resulted in a better crystal form containing only one 14-mer in the crystallographic asymmetric unit which diffracts to higher resolution. Crystallization studies of human histidyl tRNA synthetase, an autoantigen implicated in polymyositis, has resulted in two new crystal forms. Neither diffracts to high resolution and both contain many copies of the 50 kD protein subunit per crystallographic asymmetric unit. More progress has been made in our studies of the DNA "polymerasome" from T4 phage. We have determined and refined the structures of three forms of the 5'→3' exonuclease ("RNase H"). The protein has structural features in common with a variety of nuclease and recombinases, but possesses a unique, new folding topology. Crystallization

efforts are continuing on other members of this system including the polymerase and helicase. Two crystal forms and native and heavy-atom derivative data sets have been obtained for the small GP 59 helicase assembly factor. Our retroviral work has focused in two areas. Several forms of retroviral integrases have been expressed, purified and tested for crystallization. And several members of the host cell NFkB family, including p65-rel, I kappa-B and BCL-3 have likewise been expressed, purified and tested for feasibility in crystallization.



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